Proteomic analysis of clathrin interactions in trypanosomes reveals dynamic evolution of endocytosis

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Suplemental information

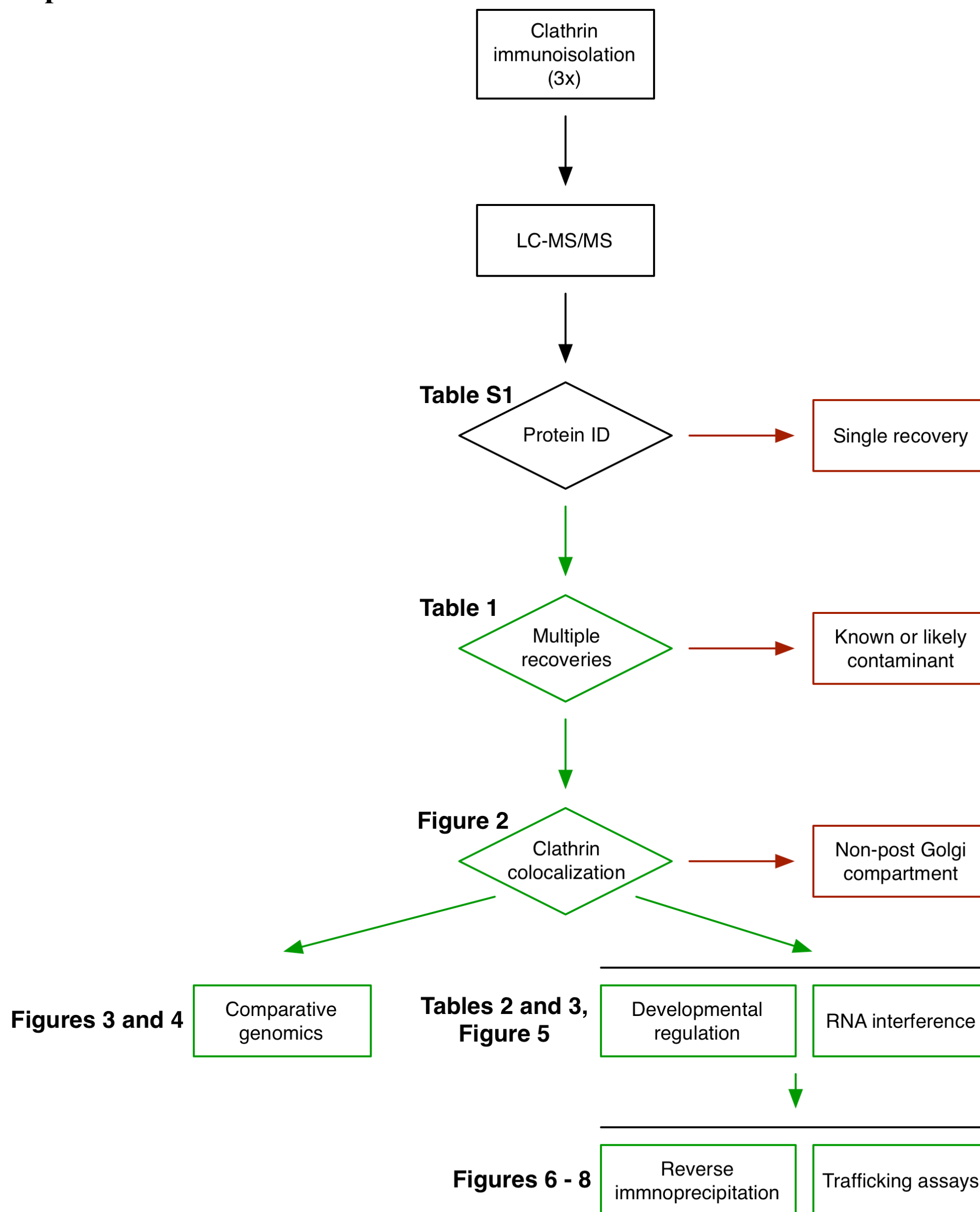


Figure S1. Experimental strategy. Flow chart describing the strategy designed for the identification and functional validation of TbCAPs. Black boxes denote the initial entry analysis. Green arrows and boxes denote that data met the criteria and the next set in analysis. Red specifies the criteria required for a fail and for halting the analysis. Callouts are given to the display items.

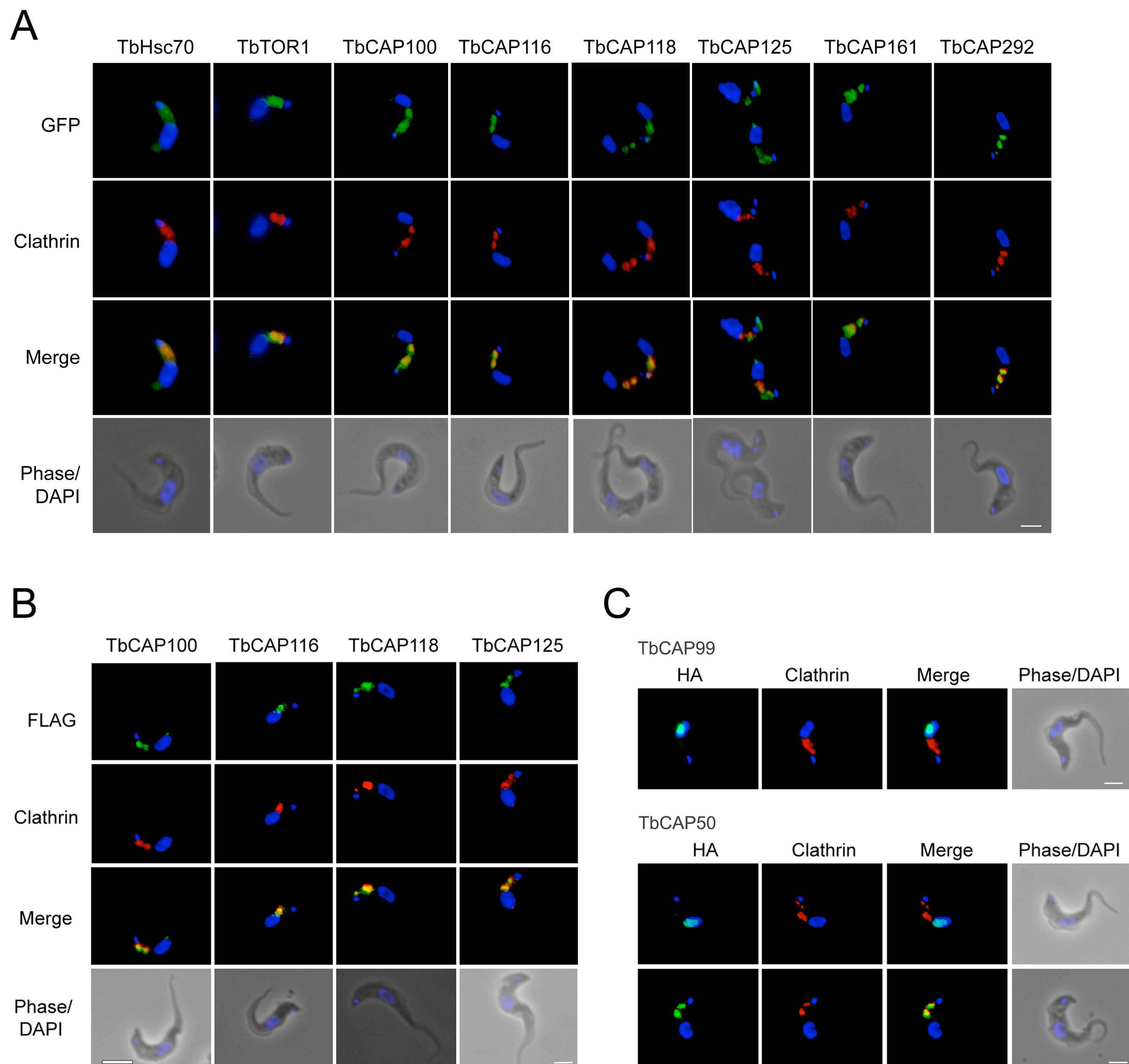


Figure S2. Immunofluorescence and co-localization of selected TbCAPs using an additional tag. Panel A: Ectopic expression of candidates fused to a C-terminal GFP tag in pXS5 vector or endogenous-locus tagged at the C-terminus. Correct integration was confirmed by Western blot or PCR. Subsequently, immunofluorescence localization of tag (green), clathrin (red) and DAPI (blue) performed. Panel B: As in A, but FLAG-tagged versions of selected TbCAPs were generated. Panel C: Two TbCAP candidates that showed unexpected localizations, inconsistent with predominant clathrin interaction. The HA-tagged gene product of Tb927.5.2660 (TbCAP99) localized to the nucleus while different clones for Tb927.7.4960 (TbCAP50) showed either nuclear or endocytic region localization. TbCAP50 was also of low molecular weight, compared with the analysed regions of the gel. These two ORFs were not pursued further. Scale bar = 2uM.

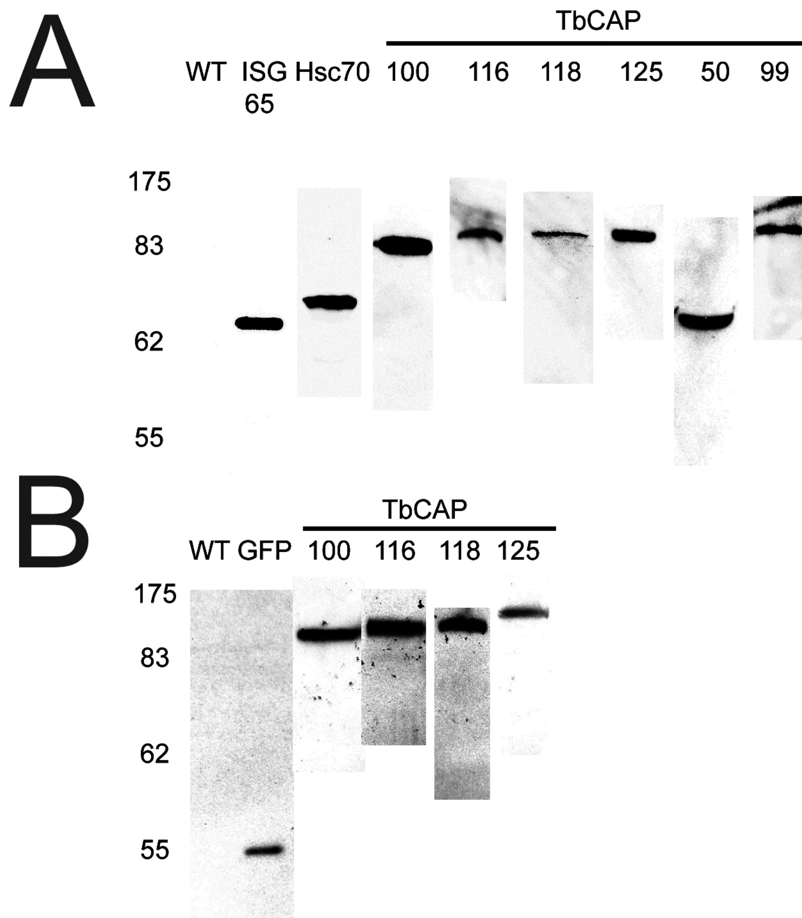
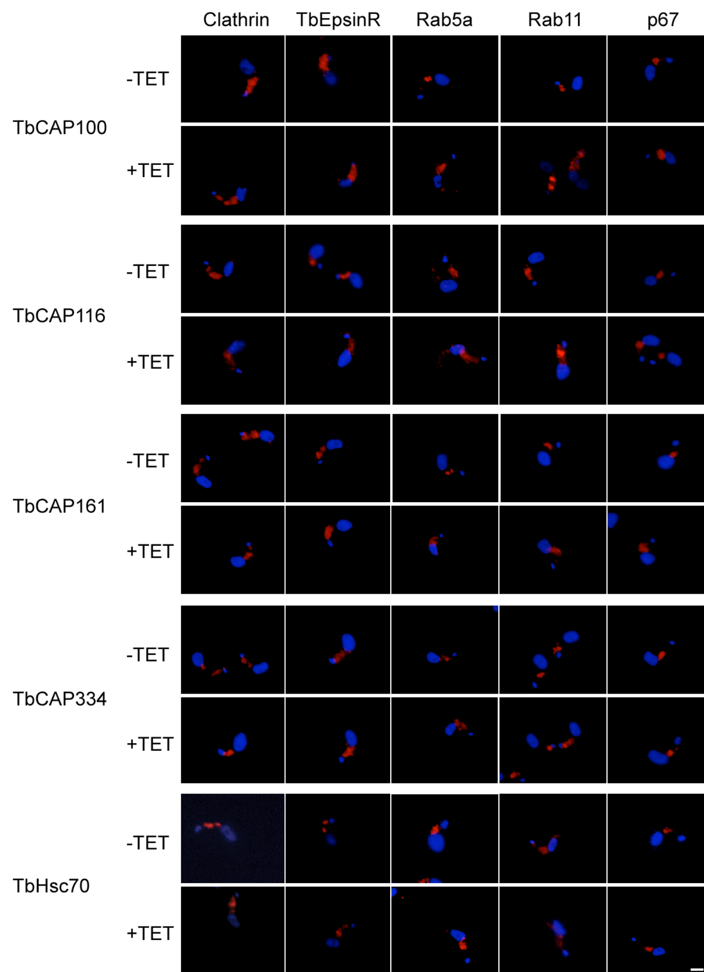


Figure S3: Western Blotting of epitope tagged TbCAPs. Panel A: Lysate from cells expressing haemagglutinin (HA) tagged TbCAPs probed with monoclonal anti-HA antibody. Wild type (WT) and HA-tagged ISG65 were used as negative and positive controls. Panel B: GFP protein as a control for Western blotting of GFP epitope-tagged cell lines. Note that the image is assembled from multiple blots which have been aligned for illustrative/clarity purposes only and that the background has been enhanced to make this obvious.



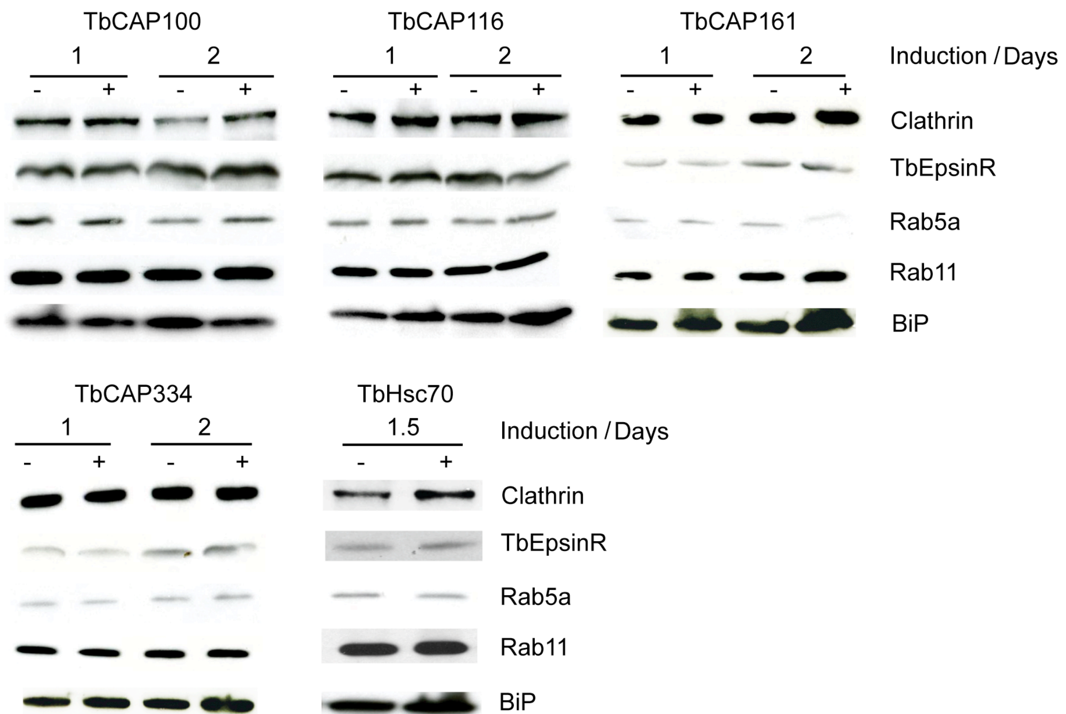


Figure S4. Immunofluorescence and Western blot analysis of endocytic markers following knockdown of selected TbCAPs. Panel A. Induced and non-induced RNAi lines were prepared for

immunofluorescence and stained with TbCHC, TbEpsinR, TbRab5A, TbRab11 and p67. No significant localization alterations were observed on depletion of the TbCAPs. Panel B. Total protein lysate of aliquots of induced and non-induced lines were probed for TbCHC, TbEpsinR, TbRab11 and TbRab5A. Equivalent loading was ensured by probing the same blots with anti-TbBiP. Scale bars = 2uM.

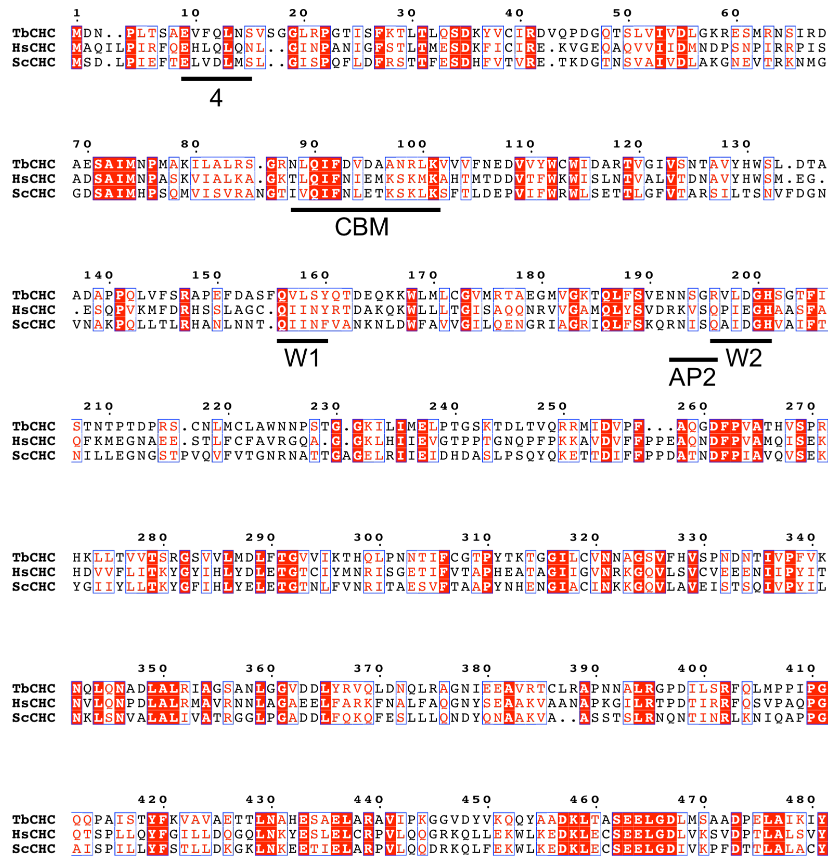


Figure S5. Alignment of *H. sapiens*, *S. cerevisiae* and *T. brucei* clathrin heavy chain protein sequences. The alignment was created in Clustal and is colored with fully conserved residues in red background, and conservative changes in red. Regions of conservation are boxed. “.” indicates gaps introduced in the alignment. Only the N-terminal 480 residues, which corresponds roughly to the β-

propeller domain are shown. Amino acids contributing to binding sites mapped by Willox and Royle (2012) for interacting protein partners in metazoan cells are underlined.