

1 **Supplemental Figure Legends**

2

3 **Supplemental Figure 1. Isoform-specific epitope tagging of trypanosomal adenylate cyclases**

4 (A) Western blot analysis of whole cell lysates from 2913 control cells and 2913 cells in which an HA
5 epitope tag was integrated into the genomic locus of ACP1, 2, 4 or 5. Blots were probed with anti-HA
6 (top) or anti-tubulin (bottom) antibodies. (B) Southern blots using genomic DNA prepared from 2913
7 control cells, or cells with an HA epitope integrated into the indicated ACP1, 2, 4 or 5 genomic locus.
8 DNA was digested with the indicated restriction enzymes and blots were probed with probes specific to
9 the ACP1 3' UTR (3' UTR) or the coding sequence of the puromycin resistance gene (Puro). The ACP1
10 UTR probe hybridizes to two bands in the tagged line, one corresponding to the untagged allele, which is
11 also observed in 2913 cells, and one corresponding to the tagged allele, which is specific to the tagged
12 line. The puroR probe does not hybridize to control (2913) DNA and hybridizes to a single band in each
13 tagged line that corresponds to the size expected for integration at the corresponding locus. (C)
14 Diagrams show restriction maps for the ACP1 locus and the indicated HA-tagged genes, as determined
15 based on the *T. brucei* 427 genomic sequence in the TriTryp genome database (91). Restriction enzymes
16 correspond to those used for the Southern blots in Figure 3, and are EcoRV (E), Nde1 (D), Mlu1 (M),
17 Nco1 (N), Sal1 (S), and BamH1 (B). The green and red bars indicate the position of probes corresponding
18 to the ACP1 3'UTR (green) and PuroR coding sequence (red), and the size of restriction fragments
19 recognized by these probes are shown below the restriction map.

20

21 **Supplemental Figure S2. Myc-tagged ACP1 is not precipitated by anti-HA antibody.** Cells expressing
22 Myc-tagged ACP1 were subjected to immunoprecipitation with anti-HA antibody. The input (I),
23 unbound (U) and bound (B) fractions were analyzed by Western blot with anti-HA or anti-Myc
24 antibodies.

25

26 **Supplemental Figure S3. Trypanosomal adenylate cyclases localize to flagellum subdomains.**

27 Images show additional examples of immunofluorescence on trypanosomes expressing the indicated
28 HA-tagged adenylate cyclases, as described in Figure 4. Cells were stained with anti-HA antibodies
29 (green), and nuclear and kinetoplast DNA were visualized with DAPI (blue).

30

31 **Supplemental Figure S4. C-terminal sequences are required for targeting to the flagellum.**

32 Images show additional examples of immunofluorescence on trypanosomes expressing the indicated
33 HA-tagged adenylate cyclase truncations, as described in Figure 6. Cells were stained with anti-HA
34 antibodies (green), anti-PFR antibodies (red), and nuclear and kinetoplast DNA were visualized with DAPI
35 (blue).

36

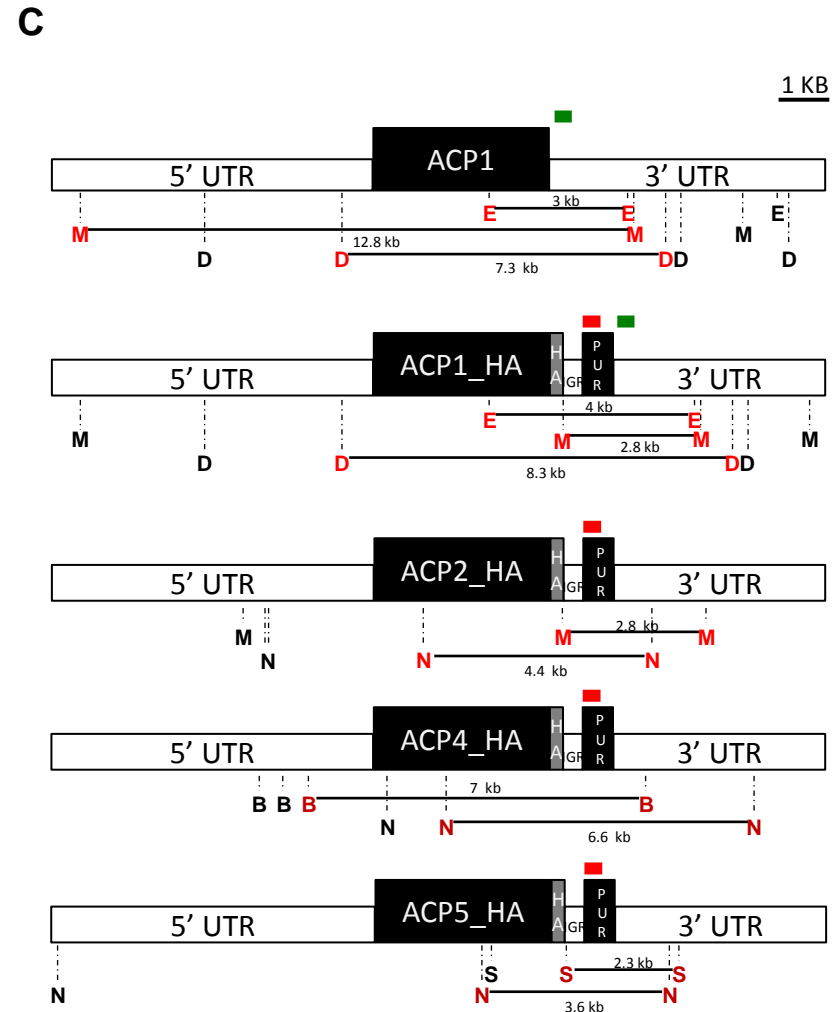
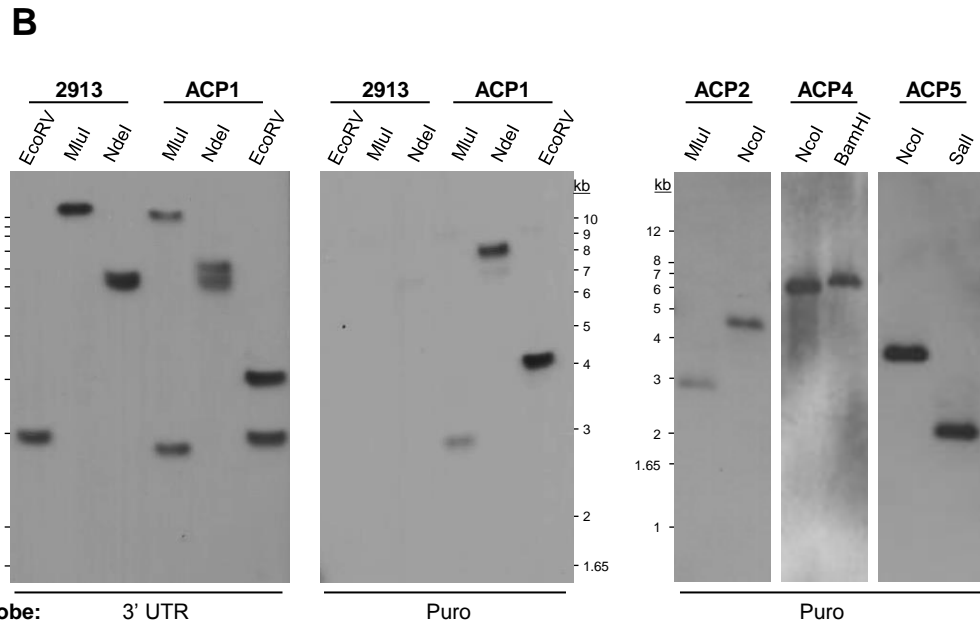
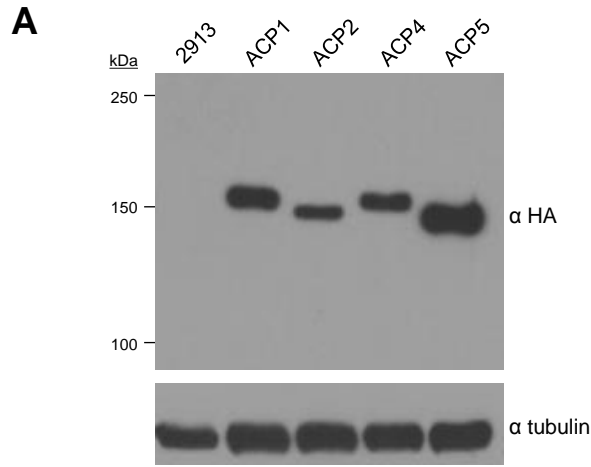
37 **Supplemental Table 1. *T. brucei* adenylate cyclases identified in the present study.**

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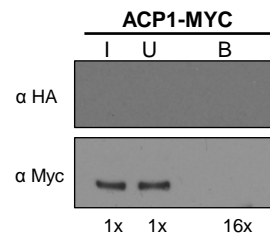
39 **Supplemental Table 2. Proteins identified through MudPIT analysis of flagellum preparations.**

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Supplementary Figure S1. Isoform-specific epitope tagging of trypanosomal adenylate cyclases



Supplementary Figure S2. Myc-tagged ACP1 is not precipitated by anti-HA antibody



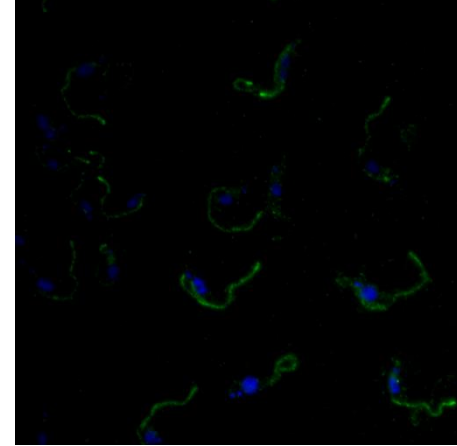
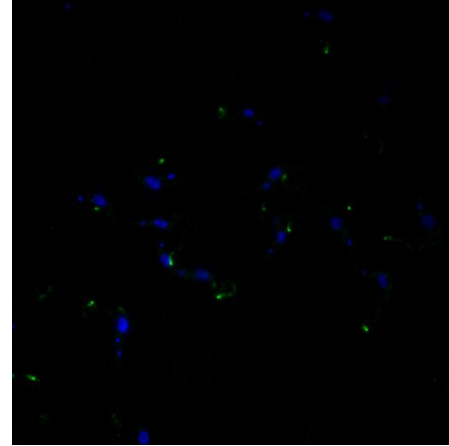
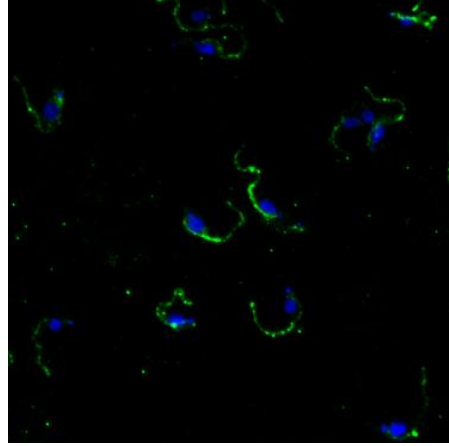
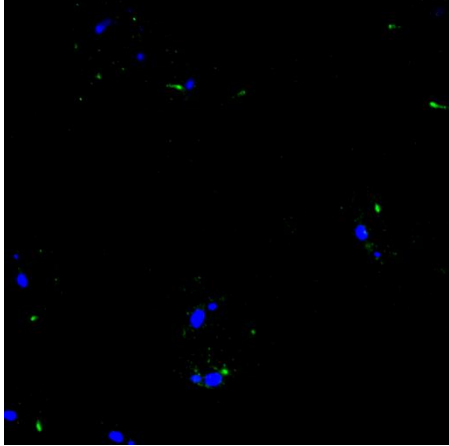
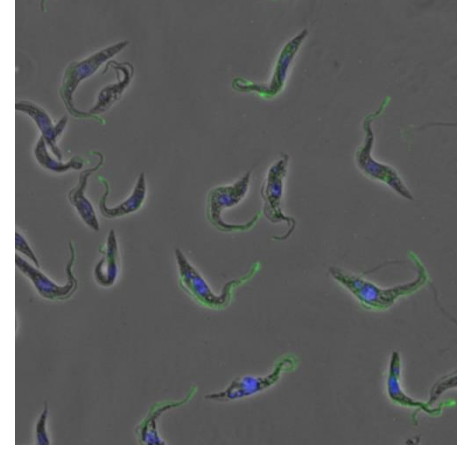
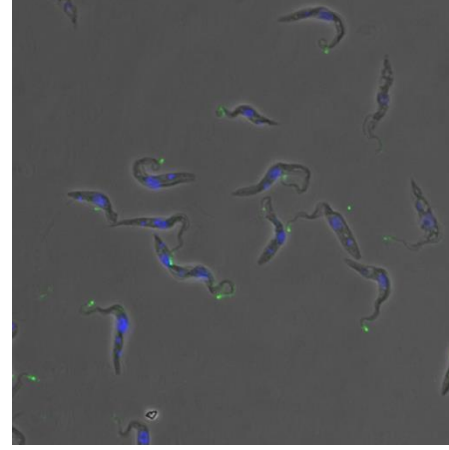
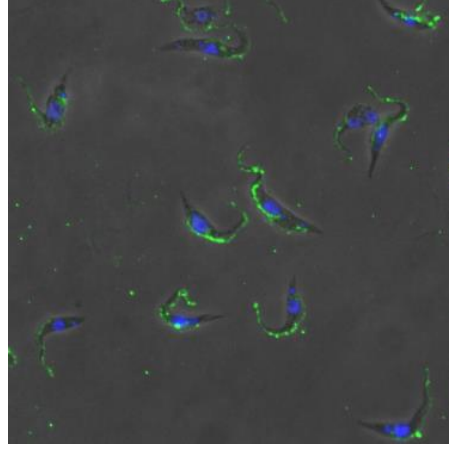
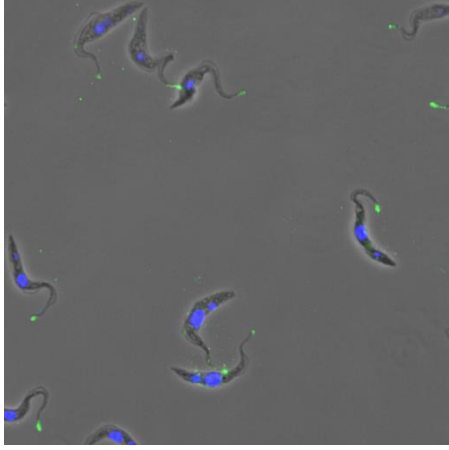
Supplementary Figure S3. Trypanosomal adenylate cyclases localize to flagellum subdomains.

ACP1

ACP2

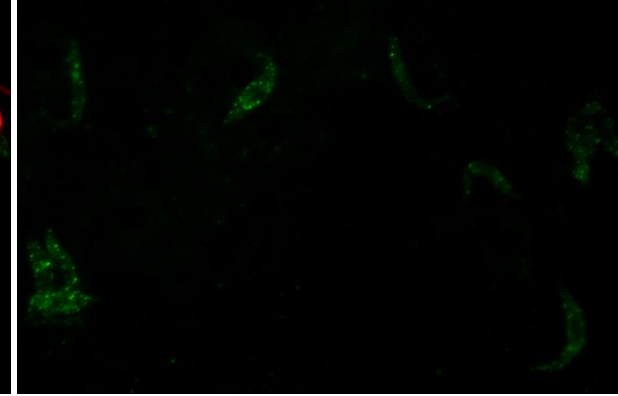
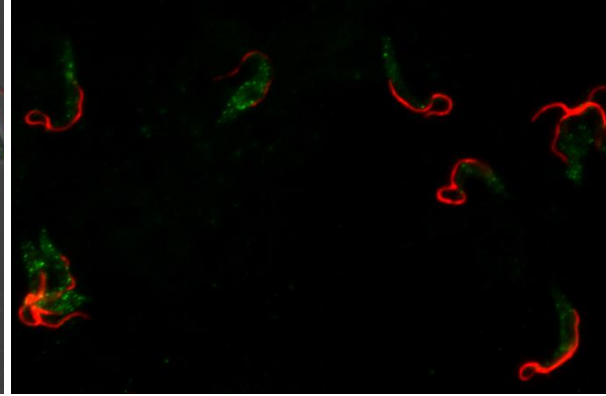
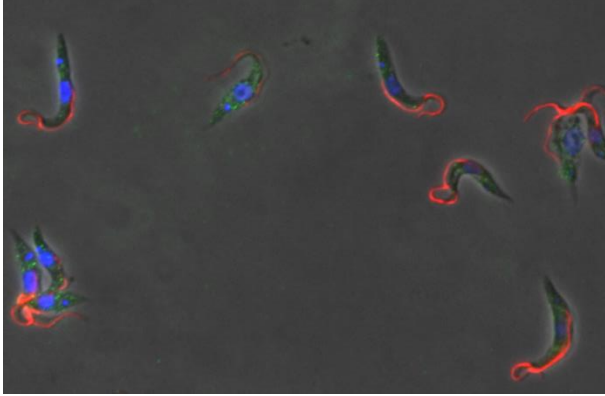
ACP4

ACP5

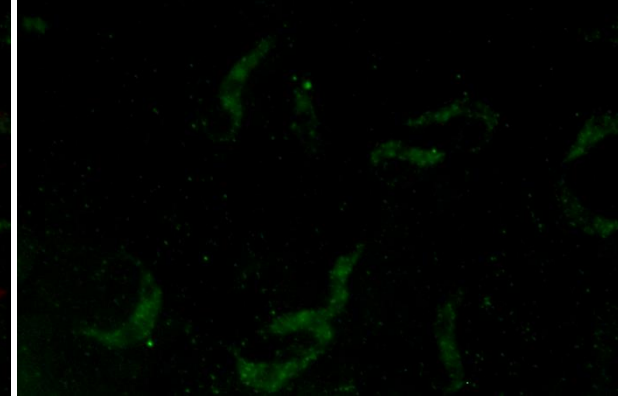
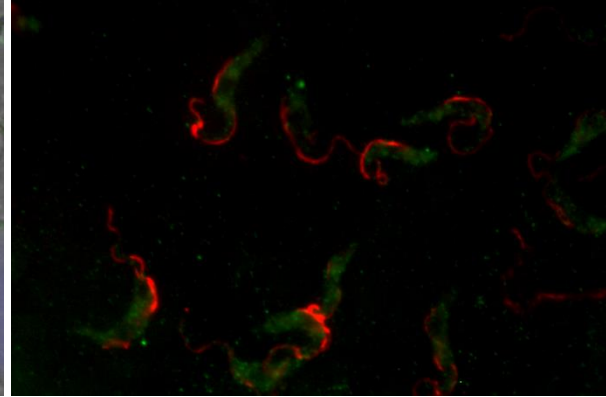
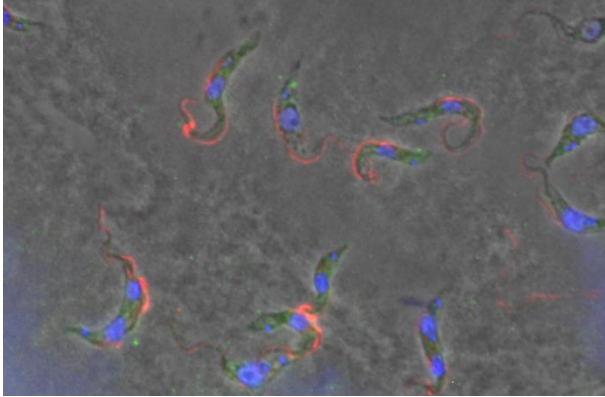


Supplementary Figure S4. C-terminal sequences are required for targeting to the flagellum

ACP1 Δ C45



ACP2 Δ C46



Supplemental Table 1.

T. brucei adenylate cyclases identified in the present study.

<u>Protein</u>	<u>GeneID</u>	<u>A.A.</u>	<u>Predicted kDa</u>	<u>Peptides Identified</u>	<u>Relatedness*</u>
AC-P1	Tb927.11.17040	1253	137.5	R.YETLPEDFIK.E, R.ADPAAETLALIR.Y, R.YVINDIVIGDYGGTCEGEAAK.H, R.TESAANGGQILLTR.A, K.EQLELETDQNK.I, R.ALISQYECYEVK.T	ESAG4-Like
AC-P2	Tb927.10.16190	1254	137.9	R.ADPAAETLALIR.Y, R.YVINDIVIGDYGGTCEGEAAK.H, R.TESAANGGQILLTR.A, K.EQLELETDQNK.I, R.ALISQYECYEVK.T	ESAG4-Like
AC-P3	Tb927.7.7470	1205	132.9	R.NYLTEYK.E, R.SLIENYDCYEVK.T, R.GVSEPVEVYQLNAVPGR.S, K.SASAWDDSYCEEVVR.R, K.EALEEANAPFVPR.R, R.SLIENYDCYEVK.T	GRESAG 4.2/4.3
AC-P4	Tb927.10.13040	1208	133.3	R.TAFMESLYEQR.R, K.GYDYYGQVPNLAAR.T, R.EEFDVTPLGEVPLR.G, R.VIDVLEEGGDGTGTSGSDR.A, K.EIAEGYR.L, K.IGTSAVSADGEDDDVDR.L, R.VIDVLEEGGDGTGTSGSDR.A	GRESAG 4.2/4.3
AC-P5	Tb927.11.13740	1214	133.8	K.SFEFVEK.L, R.TGQVIFSGTNPLACDTEYK.A, R.YVVDLVIGDYGNECGEEAIR.Q, R.VDNLNSVVPDR.S, R.EYNPPTAYLDSEVYSR.L, R.TESVANGGQVLMTR.G, R.GVPEPVEMYQLDAVAGR.V	
AC-P6	Tb927.9.15660	1242	137.5	K.DGTVGLAALR.C, R.TESIANGGQVLLTR.A, R.GVPEPVEIYQLDAVPGR.T	

* based on GeneID assignments in Salmon *et al* (15)