Supplementary Figure Legends

Supplementary Figure S1. Regions of AC coding sequences targeted for RNAi and utilized for qRT-PCR. Schematic illustration of AC domain structure with regions of each AC used for RNAi (red lines) or qRT-PCR (blue lines) indicated. The AC1&2 RNAi fragment targets both AC1&2, all other RNAi and all qRT-PCR fragments are unique. Schematics are roughly to scale with approximate nucleotide position indicated at top.

Supplementary Figure S2. AC6 knockdown targeting the AC6 open reading frame does not affect growth in suspension. (A) Relative mRNA abundance for the indicated AC genes was determined by qRT-PCR in the corresponding knockdowns grown in the absence or presence of tetracycline to induce RNAi. For AC1&2 dual KD, AC1 and AC2 mRNA abundance were determined separately. For other knockdowns, expression of the gene targeted by knockdown is shown. Error bars show standard deviation. (B) Cumulative growth curves for the indicated AC knockdown lines grown in the absence or presence of tetracycline.

Supplementary Figure S3. **Knockdown of AC genes does not affect parasite motility in suspension.** Motility was examined for 2913 control cells or the indicated AC knockdown lines maintained in the absence or presence of tetracycline to induce RNAi as indicated. P value for students unpaired t-test is shown in the one case where a significant difference was observed between –Tet and +Tet.

Supplementary Figure S4. AC6 RNAi knockdown targeting the 3'UTR does not affect growth or motility of individual cells. (A) AC6 mRNA abundance was determined by qRT-PCR in AC6-uKD cells maintained in the absence or presence of tetracycline to induce RNAi. Error bar shows standard deviation. (B) Growth curves and (C) motility of individual cells maintained in suspension culture with or without tetracycline.

Supplementary Figure S5. RNAi is gene-specific. Abundances of the indicated mRNAs were determined by qRT-PCR. (A) Abundances of AC1, AC2, AC3 and AC5 in the AC6-uKD line grown with or without tetracycline to induce RNAi as indicated. (B) Abundance of AC6 mRNA in the AC1&2 dual KD grown with or without tetracycline as indicated. AC6 is the most similar to AC1 and 2 DNA sequences (44). (C) Abundance of AC6 mRNA in the AC5-KD grown with or without tetracycline as indicated. Error bars show standard deviation.

Supplementary Figure S6. Growth curves and motility of individual cells in suspension culture for AC6-Ri and AC6**-Ri. The indicated cell lines maintained in the absence or presence of tetracycline to induce RNAi and analyzed for growth (A) and motility (B) in suspension cultures.

Supplementary Figure S7. Total cellular cAMP levels were determined for the indicated cell lines grown in the absence or presence of tetracycline to induce RNAi as indicated. Error bars indicate standard deviation

nucleotides

















A _{1e18}] AC6**Ri 1e18 AC6Ri 1e16 1e16 1e14 1e14 Cells/ml 1e12 1e12 1e10 1e10 — -Tet ----- +Tet 1e08 1e08 — -Tet +Tet 1e06 1e06 2 4 6 Days post-induction 4 6 Days post-induction 0 2 10 2 10 8 0 8 В 6-6₁ AC6** Ri AC6 Ri Avg Velocity (µm/s) 5-5 o 4 4 00 0 3. 3 00 00 ۰^۵ 2-2 1 0 0 -tet +tet -tet +tet

