SUPPLEMENTAL MATERIAL

Experimental procedures.

DNA extraction. DNA was extracted from 10^6 *T. brucei* TC221 cells by the following procedure. The trypanosomes were centrifuged at 3,000 g for 3 min and resuspended in 100 µl of 50 mM Tris-HCl pH 7.6 supplemented with 1 mM dithiothreitol (DTT). The sample was incubated for 5 min at 100°C, precipitated by adding an equal volume of isopropanol, and centrifuged for 5 min at 16,000 g. The pellet was washed with 70% (v/v) ethanol, recentrifuged and resuspended in 100 µl of 50 mM Tris-HCl pH 7.6.

Isolation of the *T. brucei* TK gene from a cDNA library. A 310-bp fragment of the TK gene was amplified from *T. brucei* TC221 genomic DNA with the following two primers:

- *TKdir: 5´-AAT TGA TCC GCA CGA CAG -3´*
- *TKrev: 5´-AAT GAC GTC GTA GTC ACG -3´*

The PCR was performed with a mixture of Taq and Pfu DNA polymerases (2.5 units of each) using 30 PCR cycles and an annealing temperature of 50°C. A partial cDNA from a putative TK of *T. b. rhodesiense* found in GenBank™ (accession no. AA0986595) was used to design the primers (the project was started before the sequencing of the *T. brucei* genome). After verification by dideoxy sequencing, the PCR product was purified on low-melting agarose, labeled with ^{32}P-dCTP using Klenow fragment and random hexanucleotide primers, and used as a probe to recover the TK ORF from a procyclic *T. brucei* 427 (TbGARP16) cDNA library.

Identification and sequencing of the two TK alleles in *T. brucei* TC221 cells. A PCR (25 cycles) was performed on a *T. brucei* TC221 lysate using the two primers, Tkdir (see above) and 5´-TTC AGT TTG CAC CAT ATT C-3´ (TKr6), with a mixture of Taq and Pfu polymerases (2.5 units of each) and an annealing temperature of 52°C. The PCR product was sequenced and confirmed to contain both 89-bp fragments. At four positions, the TK sequence was ambiguous due to the presence of two different alleles (see Results). A third PCR (25 cycles, annealing temperature 50°C) was performed on the trypanosome lysate with Tkdir as the 5´-primer. The other primer was either 5´-CAG AAC GCG GCC ACA C-3´ (TKrev1) or 5´-CAG AAC GCG GCC ACA T-3´ (TKrev2), which are identical except for the 3´-base. These two primers could discriminate between G and A at the fourth ambiguous position. The PCR product with the primers TKdir and TKrev1 was found to have an identical sequence to the one obtained from the cDNA library whereas the PCR product with Tkdir and TKrev2 differed at the four ambiguous positions. Finally, a PCR was performed with the primers 5´-CCA CAC GCT CGC ATT-3´ (TKf3) and PCR-4 (see sequence below) to also cover the region downstream of TKrev1 and TKrev2. Dideoxy sequencing of the PCR product with the allele-specific primers 5´-GCT CAA AGA TCA TGT TAT-3´ and 5´-GCT CAA AGA TCA TGT TAC-3´ revealed a fifth allelic difference (see Results).

Creation of a pET expression vector containing His_6-tagged *T. brucei* TK. The *T. brucei* TK open reading frame (ORF) was amplified from pUC-*Tb*TK (the plasmid is described in the main text) using

forward and reverse primers containing restriction sites for NdeI and BamH1, respectively (underlined):

- *TbTK-pET: 5´-ATT GCC ATC TCC GTC GTG CAT ATG CAC GAC GGA GAT GGC AAT-3´*
- *PCR-4: 5´-TAG CTG GAT CCT GGC ACA ATA CGC TGC G -3´*

The PCR product was digested by NdeI and BamHI and subcloned into a pET3a vector opened by the same enzymes. The resulting plasmid was called pET-*Tb*TK. In order to make a His_6-tagged *T. brucei* TK construct, two oligonucleotides were constructed:

- *His-tag NdeI fwd: 5´-TAT GCA TCA CCA TCA CCA TCA CGC-3´*
- *His-tag NdeI rev: 5´-TAG CGT GAT GGT GAT GGT GAT GCA-3*

The two oligonucleotides were annealed and ligated into pET-*Tb*TK opened by NdeI to create the plasmid pET-His*Tb*TK.

Creation of expression vectors encoding fusion proteins attached to full-length *T. brucei* TK, domain 1, domain 2 or human TK1. The full-length *T. brucei* TK and each of its two domains were amplified from pET-*Tb*TK by PCR using forward and reverse primers containing restriction sites for NcoI and Acc65I, respectively (underlined). For the amplification of human TK1, a cDNA clone (IMAGE 2905608, Source Bioscience, UK) was used as template and the forward primer contained a BspH1 site (isoschizomer of NcoI). The primer pairs used were the following:

Domain 1:

- *TKD1R1_Fwd-NcoI: 5´-AAC TGC TCC ATG GCA ATG CAC GAC GGA GAT GGC A-3´*
- *TKD1R1_Rev-Acc65I: 5´-GGA CCG GTA CCT TAA CCG TGT GCA CCA TTT GGC AC-3´*

Domain 2:

- *TKD2_TrxA Fwd-NcoI: 5´-AAC TGC TCC ATG GTG CCA AAT GGT GCA CAC GGT CGC-3´*
- *TKD2TrxA Rev-Acc65I: 5´-ACG GGG TAC CTA AGT AGT ATC AAC GGC CAT-3*

Full length T. brucei TK:

- *TbTKfull_fwd-NcoI: 5´-AAC TGC TCC ATG GCA ATG CAC GAC GGA GAT GGC A-3´*
- *TbTKfull_rev-Acc65I: 5´-ACG GGG TAC CTA AGT AGT ATC AAC GGC CAT-3´*

Human TK1:

- *hTK_Fwd, BspH1: 5´- GCT ACT CAT GAG CTG CAT TAA CCT GCC CAC-3´*
- *hTK_Fwd, Acc65I: 5´-GAT ACG GTA CCT CAG TTG GCA GGG CTG C-3´*

The PCR products were subsequently digested with NcoI (or BspH1) and Acc65I and subcloned into several variants of bacterial expression vectors from European Molecular Biology (see main text). They were all verified by DNA sequencing from both directions.

Expression and purification of fusion constructs containing removable His-tagged fusion partners in frame with human TK1, *T. brucei* full-length TK, domain or domain 2. Z-tagged human TK1, Z-tagged *T. brucei* TK, MBP-tagged domain 1, and TrxA-tagged domain 2 were expressed in Rosetta (DE3) pLysS *E. coli* cells (Novagen) and grown in the presence of 34 mg/L chloramphenicol and 25 mg/L kanamycin (34 mg/L chloramphenicol and 100 mg/L carbenicillin for TrxA-tagged domain 2) in 1 L Luria-Bertani broth. Growth temperatures and IPTG induction were similar as described for the His_6-tagged *T. brucei* TK. The induced cells were harvested, washed in

buffer A (0.3 M NaCl, 50 mM Na_2SO_4, 6 mM β-mercaptoethanol, and 20 mM Tris-HCl pH7.8), and split into two tubes. For domain 1 and the human TK1, Na_2SO_4 was omitted from buffer A here and in subsequent steps. For each purification, the material from one of the tubes was thawed, resuspended in 10 ml buffer A containing 10 mM imidazole, and centrifuged at 45,000 rpm for 1 hour (Beckman L-90 ultracentrifuge, Ti70 rotor). The supernatant was applied to a column containing 1 ml Nickel-NTA Superflow (Qiagen), which was subsequently washed in a step-wise manner with buffer A containing higher and higher concentrations of imidazole (10 mM, 20 mM, and 50 mM). Each washing step was continued until no more detectable protein came off the column (as measured with the Bio Rad Protein Assay). TrxA-tagged domain 2 and Z-tagged *T. brucei* TK were eluted with 150 mM imidazole whereas Z-tagged human TK1 and MBP-tagged domain 1 were purified using an additional washing step with 100 mM imidazole before elution with 250 mM imidazole. The buffer of the purified proteins was subsequently exchanged into buffer A (supplemented with 4 mM $MgCl_2$) by Sephadex G-25 chromatography. The fusion partner of each of the purified proteins was removed by digestion with equal amount (in mg) of His_6-tagged TEV protease at 25°C for 2.5 hours (no loss of enzyme activity was observed during the incubation step) and subsequently applied to a second Nickel-NTA Superflow column equilibrated with buffer A. The His_6-tagged fusion partners, TEV protease, and non-cleaved protein remained on the column, whereas the TK proteins/domains eluted mainly in the flow-through and to some extent in the subsequent 20 mM imidazole wash. The protein solutions were, if necessary, concentrated by centrifugation with a 30 kDa cutoff filter (Centricon or Amicon tubes, Millipore) and subsequently divided into aliquots for storage at -80°C. They were only thawed once to avoid any loss of enzymatic activity due to repeated freeze-thaw cycles.

SUPPLEMENTAL FIGURES S1-S6

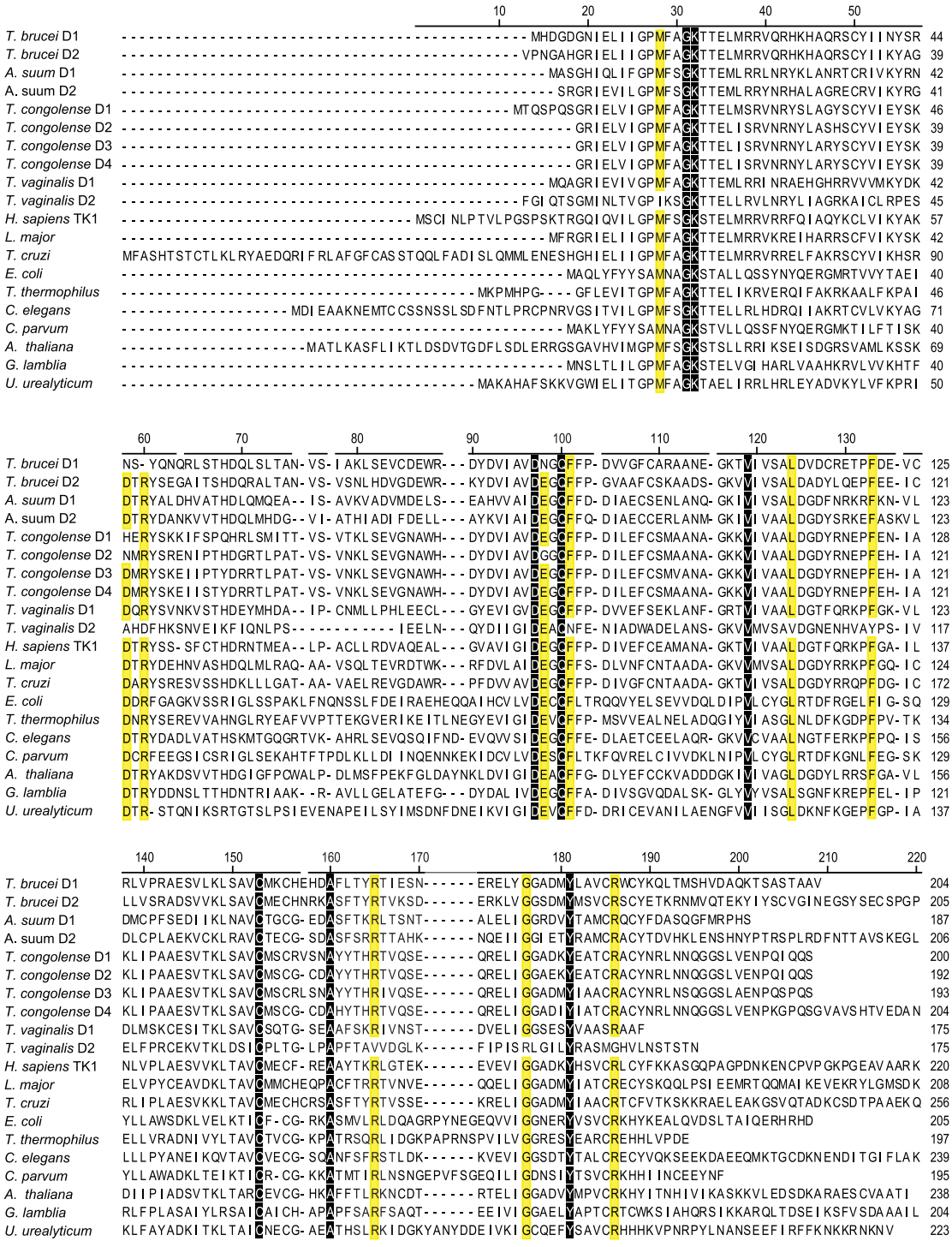

Fig. S1. Sequence alignment of the TK1 domains and proteins used for constructing the phylogenetic tree in Fig. 3B. The numbering is based on the human TK1 sequence. Conserved amino acid residues in all aligned species are highlighted in black whereas the yellow color indicates residues that differ in some domains but are conserved in all single-domain proteins. C-terminal ends of *T. brucei* (D2), *A. suum* (D2), *T. congolense* (D2), *H. sapiens*, *L. major*, *T. cruzi*, *C. elegans* and *G. lamblia* TKs are truncated in order to fit the alignment onto a single page.

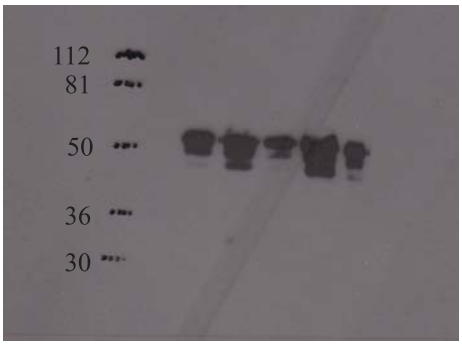

Fig. S2. Western blot analysis of different *T. brucei* TK preparations with a mouse α-His-antibody (Invitrogen) shows a major full-length TK band (52 kDa) and additional C-terminal degradation products (the His_6-tag is in the N-terminus). The upper band is the full-length TK band and was the major band in all lanes at shorter exposure times. Corresponding positions of pre-stained reference protein bands on the blotting membrane are shown to the left.

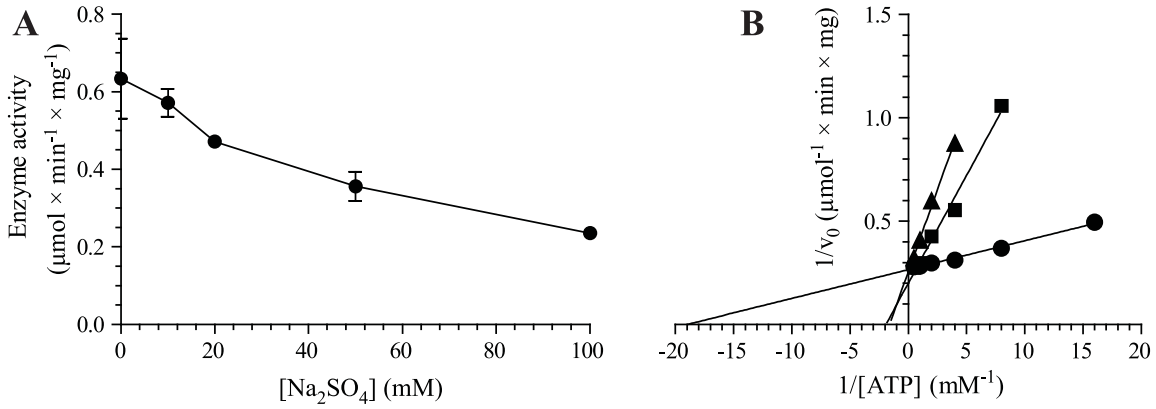

Fig. S3. Effects of sulfate on *T. brucei* TK activity. (A) Enzyme activity with 5 μM thymidine and 2 mM ATP in the presence of different concentrations of Na_2SO_4. The experiment was made in duplicate with the standard deviations indicated. (B) Lineweaver-Burk diagram showing that sulfate inhibits the TK enzymatic reaction by acting as a competitive inhibitor with respect to ATP. The experiments were performed in the absence (●) or presence of 10 mM (■) or 20 mM (▲) ammonium sulfate. The thymidine concentration was 0.5 mM in B in order to make ATP to be the limiting substrate.

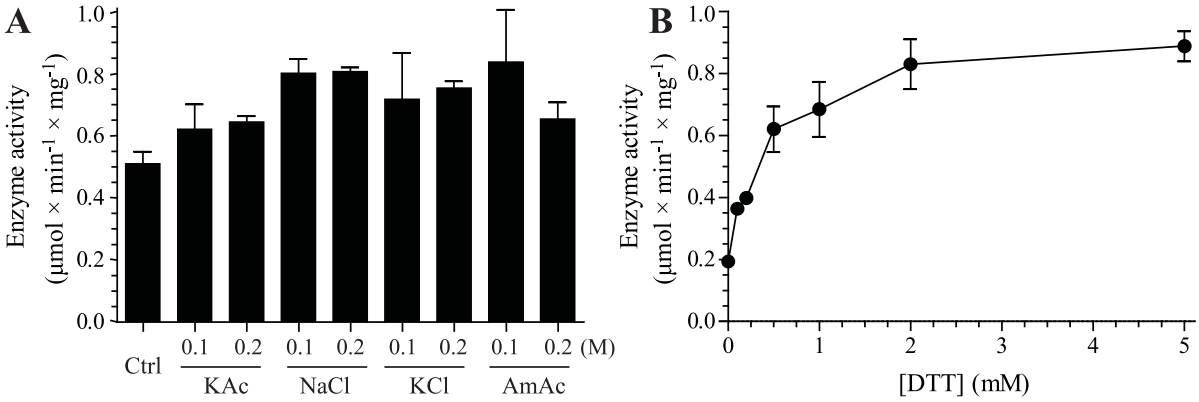

Fig. S4. Effects of salts and DTT on *T. brucei* TK activity. (A) TK enzyme activity in the absence (ctrl) or presence of different potassium, sodium or ammonium salts at 0.1 and 0.2 M concentration. (B) Effect of DTT concentration on enzyme activity. The experiments were made in duplicate with the standard deviations indicated. The substrates were 5 μM thymidine and 2 mM ATP.

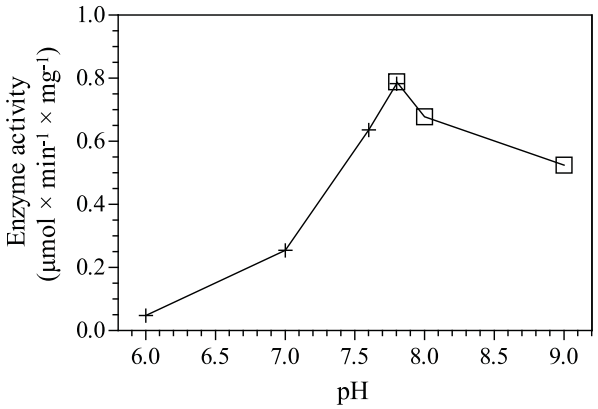

Fig. S5. Dependence of *T. brucei* TK activity on pH. The buffer used in the assays was Hepes-KOH between pH 6-7.8 (+) and Tris-HCl between pH 7.8-9 (□).The experiments were made in duplicate with the standard deviations indicated. The substrates were 5 μM thymidine and 2 mM ATP.

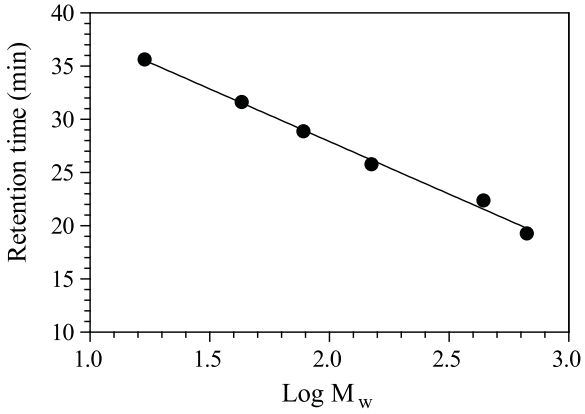

Fig. S6. Standard curve made from gel filtration analysis of myoglobin (16.9 kDa), ovalbumin (43 kDa), transferrin (78 kDa), IgG (150 kDa), ferritin (440 kDa) and thyroglobulin (670 kDa).