Supporting information for:

Inhibition of F_1-ATPase from *Trypanosoma brucei* by its regulatory protein inhibitor $TbIF_1$

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TABLE S1

Intact molecular masses of $TbIF_1$ and its variants

$TbIF_1$ variant	Mass (Da)		Mass difference	Modification
	Observed	Calculated	(kDa)	
$TbIF_1$-WT	12148	12148.6	-0.6	None
$TbIF_1$(1-64)	8608	8608.5	-0.5	None
$TbIF_1$ (Y36W)	12171	12170. 6	0.4	None
$TbIF_1$ (P32A)	12121	12121.5	-0.5	None
$TbIF_1$(E24A)	12089	12089.5	-0.5	None
$TbIF_1$(E27A)	ND	11649.0	ND	ND
$TbIF_1$-Δ1-5	11648	11199.6	-1.0	None
$TbIF_1$-Δ1-8	11493	10958.3	-0.6	None*
$TbIF_1$-Δ1-10	11199	10615.9	-0.3	None*
$TbIF_1$-Δ1-12	10958	11492.9	-0.9	None*
$TbIF_1$-Δ1-15	10615	12089.5	0.1	None*

*N-terminal methionine was retained; ND, not determined

TABLE S2

Interactions between amino acids in subunits of bovine F_1-ATPase and bovine IF_1 and their possible conservation in *T. brucei*

Bold residues are identical in bovine and *T. brucei* mitochondria. Brackets denote non-identical residues at equivalent positions in the *T. brucei* ortholog.

I1-60$_E$	**β_E**	**β_{TP}**	**β_{DP}**	**γ**	**α_{DP}**	**α_E**
E31	**R408**					
Y33	**K401**					
Q41 (T)	**D450**					
I1-60$_{TP}$						
R25 (K)				E241 (S)		
E30		**R408**				
Y33		**K401**				
F34 (A)		**E454**, S405 (D), **R408**				
Q41 (T)		**D450**				
I1-60$_{DP}$						
S11 (H)				N15 (R)		
A12 (R)						E353 (D)
G13 (K)			**D386**			
V15 (E)			**D386**			
D17			**D386**			
F22		**D386,** I390 (V), **L391**		I16 (F)		
E30			**R408**			
Y33			M393 (I), **D394**, **K401**			
F34 (A)			**V404**, S405 (D), **R408**, **E454**			
R35 (L)					E399 (K)	
Q41 (T)			**D450**			
L42 (M)			**P453**, L473 (M), **A474**, H477 (A)			
L45			**A470**, D471 (K), **A474**			

Adapted from ref (9).

TABLE S3

List of oligonucleotides

Sequence	Use
TAGCATATGCATATGAGCGAGGGGAAGCCAACTGAAGG	$TbIF_1$-WT amplification, forward primer (F)
TAGCATATGCATATGACTGAAGGACACAG	$TbIF_1$-Δ1-5 amplification F
TAGCATATGCATATGCACAGAAAGATCAAC	$TbIF_1$-Δ1-8 amplification F
TAGCATATGCATATGAAGATCAACCTGGAC	$TbIF_1$-Δ1-10 amplification F
TAGCATATGCATATGAACCTGGACGATG	$TbIF_1$-Δ1-12 amplification F
TAGCATATGCATATGGATGATGAGAGGTGG	$TbIF_1$-Δ1-15 amplification F
CGAAAGCTTGCTAGCTTAGTGATGGTGATGGTGATGTTGCTTCTCGTTCGTTAACTGC	$TbIF_1$-WT amplification, reverse primer (R)
CGAAAGCTTGCTAGCTTAGTGATGGTGATGGTGATGTTGCTTCTCGTTCGTTAACTGC	$TbIF_1$(1-64) amplification R
CTTCGGTCTCCAGAAGAACGATGGGCACTCGAACGACA	$TbIF_1$(Y36W) mutagenesis F
TGTCGTTCGAGTGCCCATCGTTCTTCTGGAGACCGAAG	$TbIF_1$(Y36W) mutagenesis R
GACGAAAAACTTCGGTCTGCAGAAGAACGATATGCAC	$TbIF_1$(P32A) mutagenesis F
GTGCATATCGTTCTTCTGCAGACCGAAGTTTTTCGTC	$TbIF_1$(P32A) mutagenesis R
GGTGGATCGAGGCGGCGTTCGACGAAAAACT	$TbIF_1$(E24A) mutagenesis F
AGTTTTTCGTCGAACGCCGCCTCGATCCACC	$TbIF_1$(E24A) mutagenesis R
GGAGACCGAAGTTTTGCGTCGAACTCCGCCT	$TbIF_1$(E27A) mutagenesis F
AGGCGGAGTTCGACGCAAAACTTCGGTCTCC	$TbIF_1$(E27A) mutagenesis R

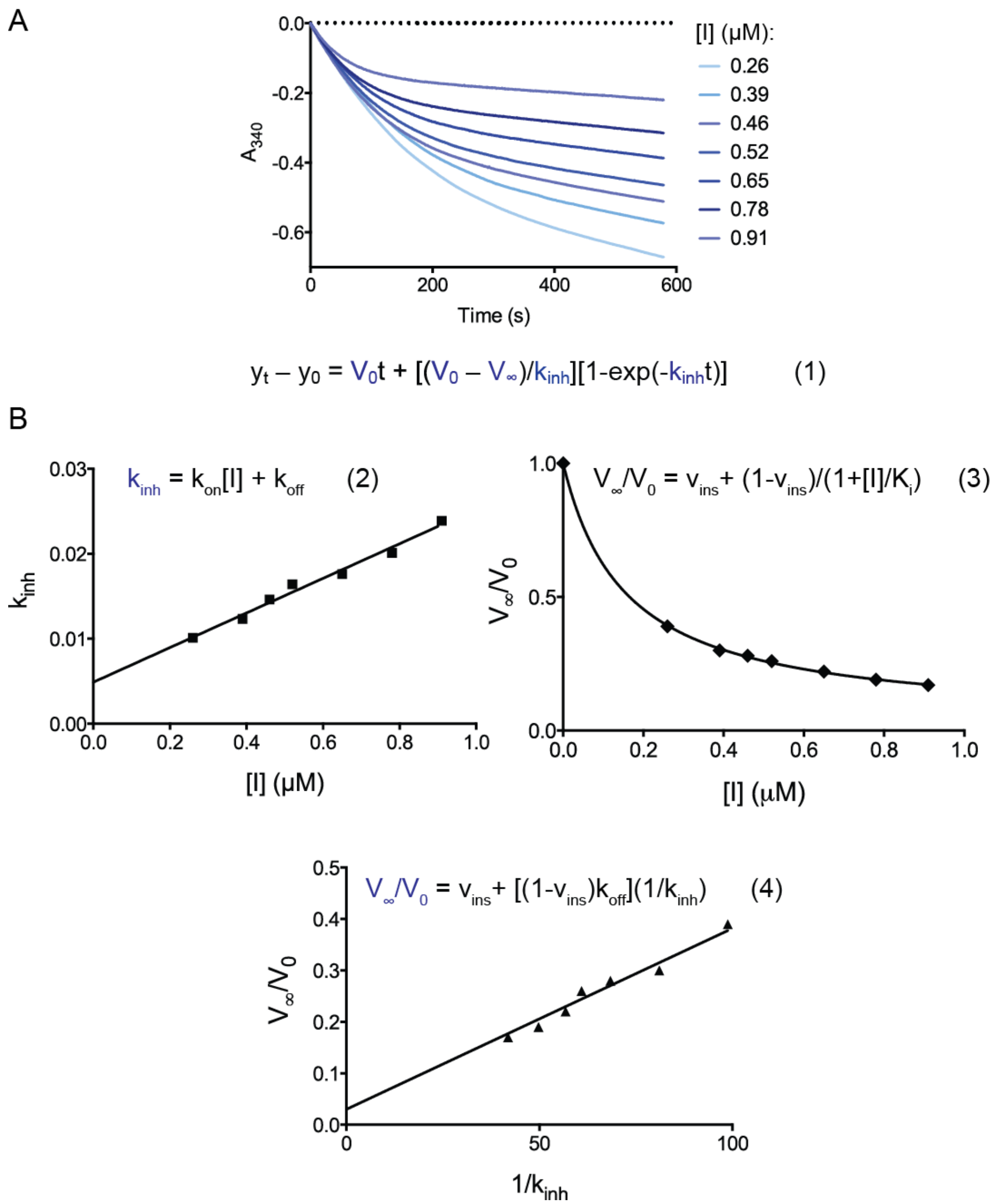

FIGURE S1. **Analysis of kinetic data illustrated with the example of TbIF$_1$-WT at pH 8.0.** (A), The decrease of NADH absorbance corresponding to the monoexponential decay of the activity of F$_1$-ATPase from *T. brucei* upon inhibition at each inhibitor concentration was fitted to equation (1) to obtain the parameters V_0, V_∞, and k_{inh}. (B), k_{on} was calculated as the slope of the linear regression of k_{inh} plotted against [I] (equation (2)). The ratio V_∞/V_0 was plotted against [I] and the data fitted to equation (3) to obtain K_i. In order to obtain k_{off}, the ratio V_∞/V_0 was plotted against $1/k_{inh}$ and data were fitted into the linear equation (4).