Supplemental Materials

Molecular Biology of the Cell

Cestari et al.

Supporting Information

Inositol polyphosphate multikinase regulation of *Trypanosoma brucei* life stage development

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2. Supporting Tables and Table legends S1-S3
3. Supporting Material and Methods
4. Supporting References

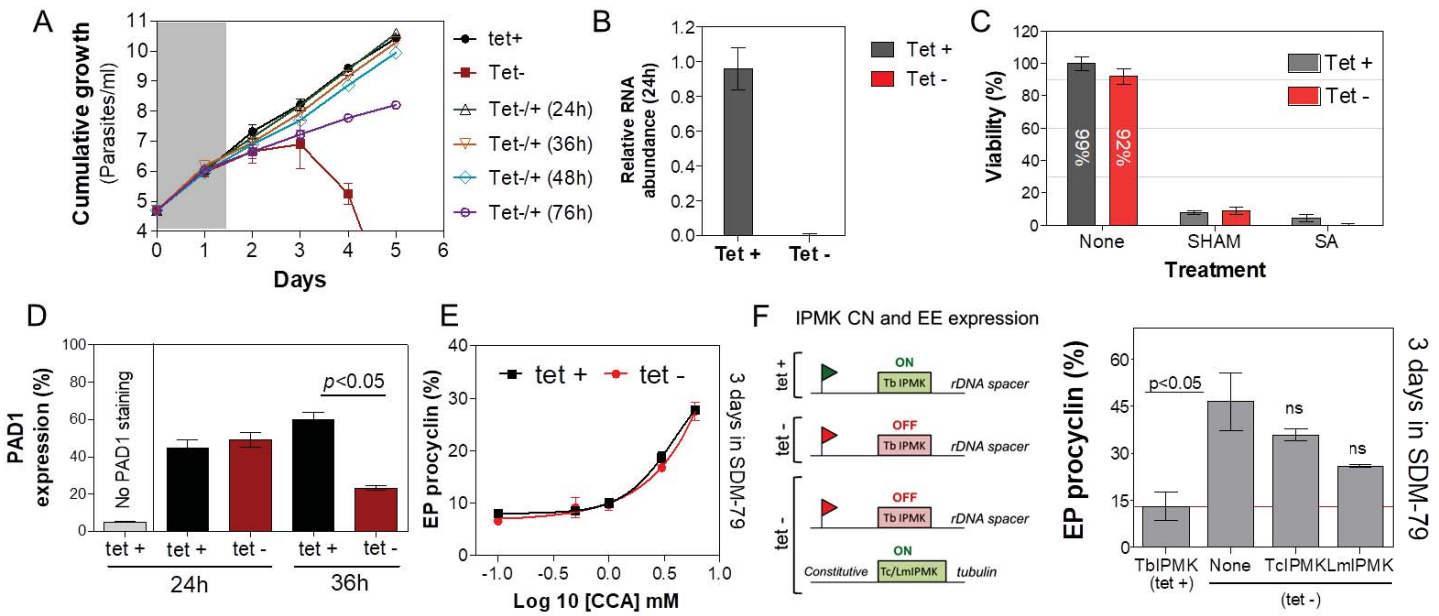

Figure S1. Growth curve, viability, EP procyclin and PAD1 expression after IPMK knockdown in *T. brucei* BFs. A) Growth curve analysis of *T. brucei* IPMK CN in BFs. The *IPMK* gene was re-expressed after 24h, 36h, 48h or 72h knockdown by adding tet to the culture (tet-/+). Growth of cells that did not receive tet (tet -) or that were always maintained in tet (tet +) are shown. Gray shading indicates 36h, the time point used for most experiments. B) IPMK mRNA knockdown after 24h. Telomerase reverse transcriptase (TERT) and β-tubulin were used as endogenous controls. Values were normalized to IPMK mRNA expression in the parental SM427 cell line. C) Viability analysis of *T. brucei* BFs IPMK CN after 36h growth in tet - (knockdown) or tet + (non-knockdown). Cells were cultured in HMI-9 at 37°C. Salicylhydroxamic acid (SHAM, 2 mM) or sodium azide (SA, 40 μM) were used to affect cell viability. "None" refers to cells not treated with any inhibitor. Viability was measured using the alamarBlue assay (1). D) Flow cytometry quantification of *T. brucei* BFs expressing PAD1 after 24h or 36h of IPMK knockdown in HMI-9 at 37°C. Cells were collected at late log phase (~1.6x10^6 parasites/ml). E) Flow cytometry quantification of cells expressing EP procyclin after tet-induced overexpression of IPMK in *T. brucei* BFs after 3 days in SDM-79 at 27°C in the absence or presence of CCA (0.5 – 6 mM). IMPK fused to a C-terminal V5 epitope tag was overexpressed using pLEW100 in SM427 strain. F) *Left*, scheme shows *T. brucei* IPMK CN that exclusively express *T. brucei* (TbIPMK), *T. cruzi* (TcIPMK) or *L.major* (LmIPMK) IPMK genes. A TbIPMK copy introduced in the rRNA spacer is regulated by tet and a copy of TcIPMK or LmIPMK is constitutively expressed from one of the tubulin loci in a derivative cell line. For details on cell line generation see (2). *Right*, flow cytometry quantification of EP procyclin expression in *T. brucei* cells that express *T. brucei* IPMK (TbIPMK, tet +), cells in which the IPMK gene was knocked down (tet -), or cells exclusively expressing either *L. major* or *T. cruzi* IPMK. *T. brucei* IPMK was knocked down in BFs growing in HMI-9 at 37°C, after which cells were transferred to SDM-79 at 27 °C and EP procyclin expression was analyzed after 3 days. Data are represented as means ± SEMs. *p*-values indicate statistical significance by the t-test; ns, not significant. For IPMK CNs, tet was used at 0.5 μg/ml, whereas for IPMK overexpression it was used at 1 μg/ml.

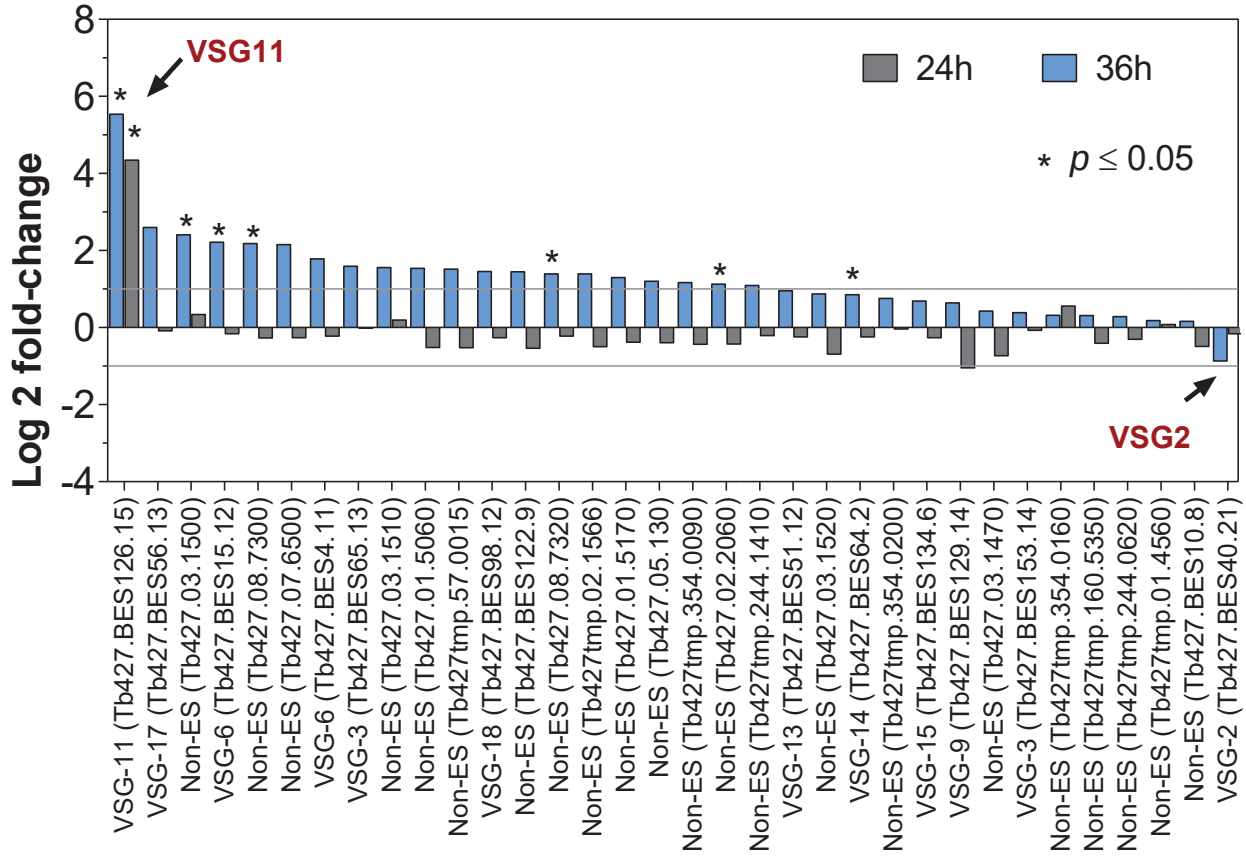

Figure S2. VSGs differentially expressed after IPMK knockdown. Analysis of VSGs differentially regulated after 24h and 36h IPMK knockdown analyzed by RNAseq. Note that at 24h and 36h there are an increase in VSG11 expression. Other telomeric and non-telomeric VSGs are also upregulated at 36h, whereas VSG2 (expressed by *T. brucei* BF IPMK CN in tet + conditions) is downregulated after knockdowns. Gray lines indicate 2-fold change (up or down); asterisks (*) indicate VSGs with *p*-value lower than 0.05. VSGs known to be in the telomeric expression sites (ES) on the parental cell line (427) (1) are indicated by their abbreviation according to GeneDB, whereas other VSGs not previously shown to be in telomeric ESs are indicated as non-ES.

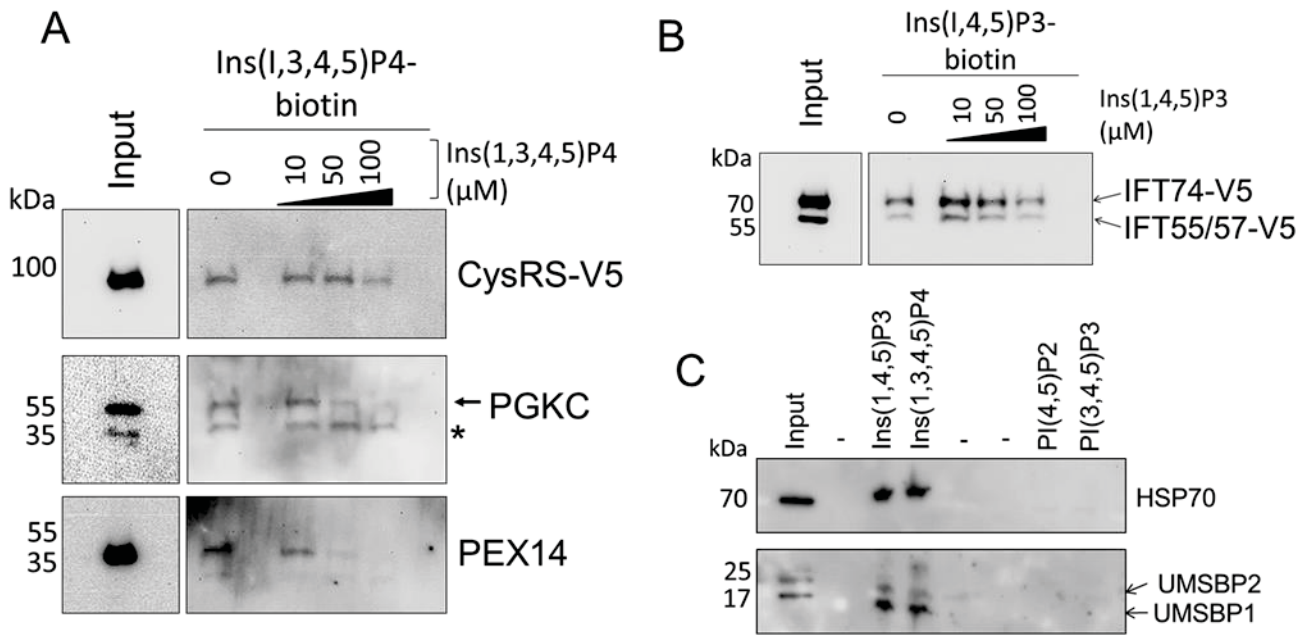

Figure S3. Western blot confirmation of IP-interacting proteins. (A-B) Western analysis of *T. brucei* proteins after affinity enrichment with (A) biotin-Ins(1,3,4,5)P4 in absence or presence of competing concentrations of free Ins(1,3,4,5)P4; or (B) biotin-Ins(1,4,5)P3 in absence or presence of competing concentrations of free Ins(1,3,4,5)P3. C) Western analysis of *T. brucei* proteins after affinity enrichment with biotin conjugated Ins(1,4,5)P3, Ins(1,3,4,5)P4, PI(4,5)P2 and PI(3,4,5)P3. Streptavidin-magnetic beads were used to capture biotin conjugated IPs/PIs. MAb α-V5 antibodies were used to detect V5-tagged CysRS, V5-tagged IFT55/57 and IFT74, mAb α-78 to detect mitochondrial HSP70, and pAb α-PGK, α-PEX14 and UMSBP to detected PGKC, PEX14 and UMSBP1 proteins, respectively. Note that IFT74-V5 is detected likely due to co-interaction with IFT55/57 (Subota, et al., 2014), and UMSBP1 is preferentially affinity enriched by both Ins(1,4,5)P3 and Ins(1,3,4,5)P4. Note that HSP70 and UMSBP1 were detected by mass spectrometry using PI(3,4,5)P3-agarose but their enrichment values did not pass the statistical cutoff and they were also not verified by Western (C). * Antibody unspecific reaction.

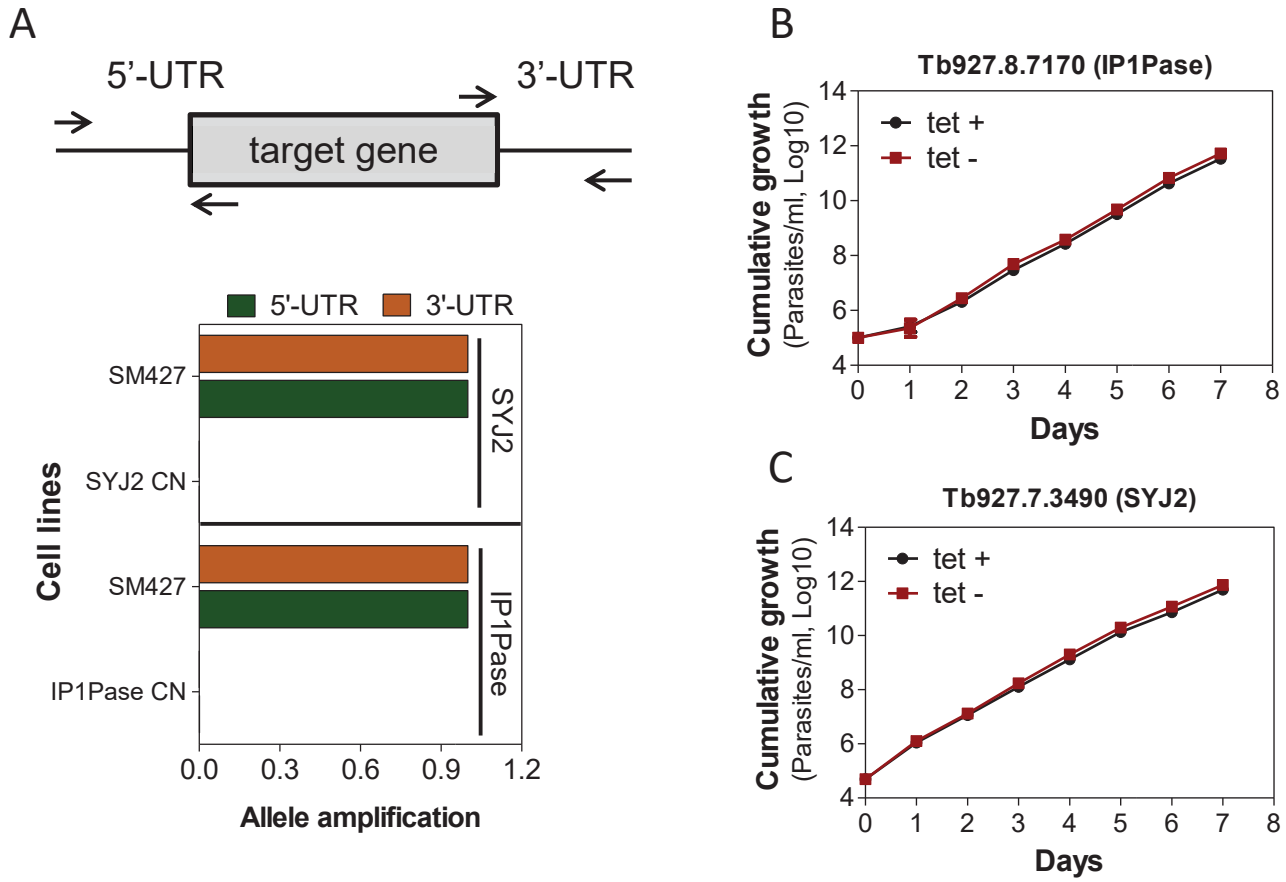

Figure S4. Genotyping and growth curves of IP1Pase and SYJ2 CNs. A) Top, diagram indicates gene target loci (SYJ2 or IP1Pase genes) with 5'-UTR and 3'-UTR. Primers (arrows) were designed to amplify a fragment of the 5-UTR or 3'-UTR with part of the gene sequences. Bottom, real-time PCR genotyping of SYJ2 or IP1Pase CNs using 5' and 3'-UTRs primers as indicated above. Note that PCR products are only amplified in the parental cell line because both alleles of the target gene were removed by homologous recombination. The resultant IP1Pase and SYJ2 CNs also express a copy of the target gene under control of tetracycline in the rDNA spacer (not indicated in the diagram). For method details see (2). B-C) Growth curve analysis of IP5Pase (B) and SYJ2 (C) in presence (expressed) or absence (knockdown) of tet (0.5 µg/ml). Data are represented as mean ± SEM.

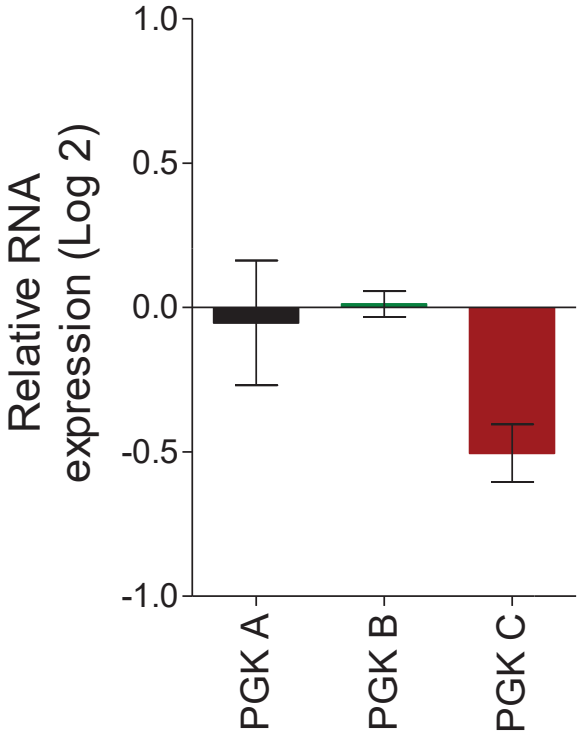

Figure S5. Real-time PCR analysis of PGK after IPMK knockdown. IPMK was knockdown for 36h in *T. brucei* BF and the expression levels of PGK A, B or C were quantified. Telomerase reverse transcriptase (TERT), β-tubulin and 18S were used as endogenous controls. Real-time PCR was used to compare relative changes of PGK genes using primers that are specific to each PGK because of the high sequence similarity between PGK genes (especially PGKB and C) which may affect differential expression analysis by RNAseq. Similarities between PGK genes, PGKA vs PGKB, 80% nucleotide (nt) identity; PGKA vs PGKC 78% nt identity; and PGKB vs PGKC 95% identity. Data are represented as mean ± SEM.

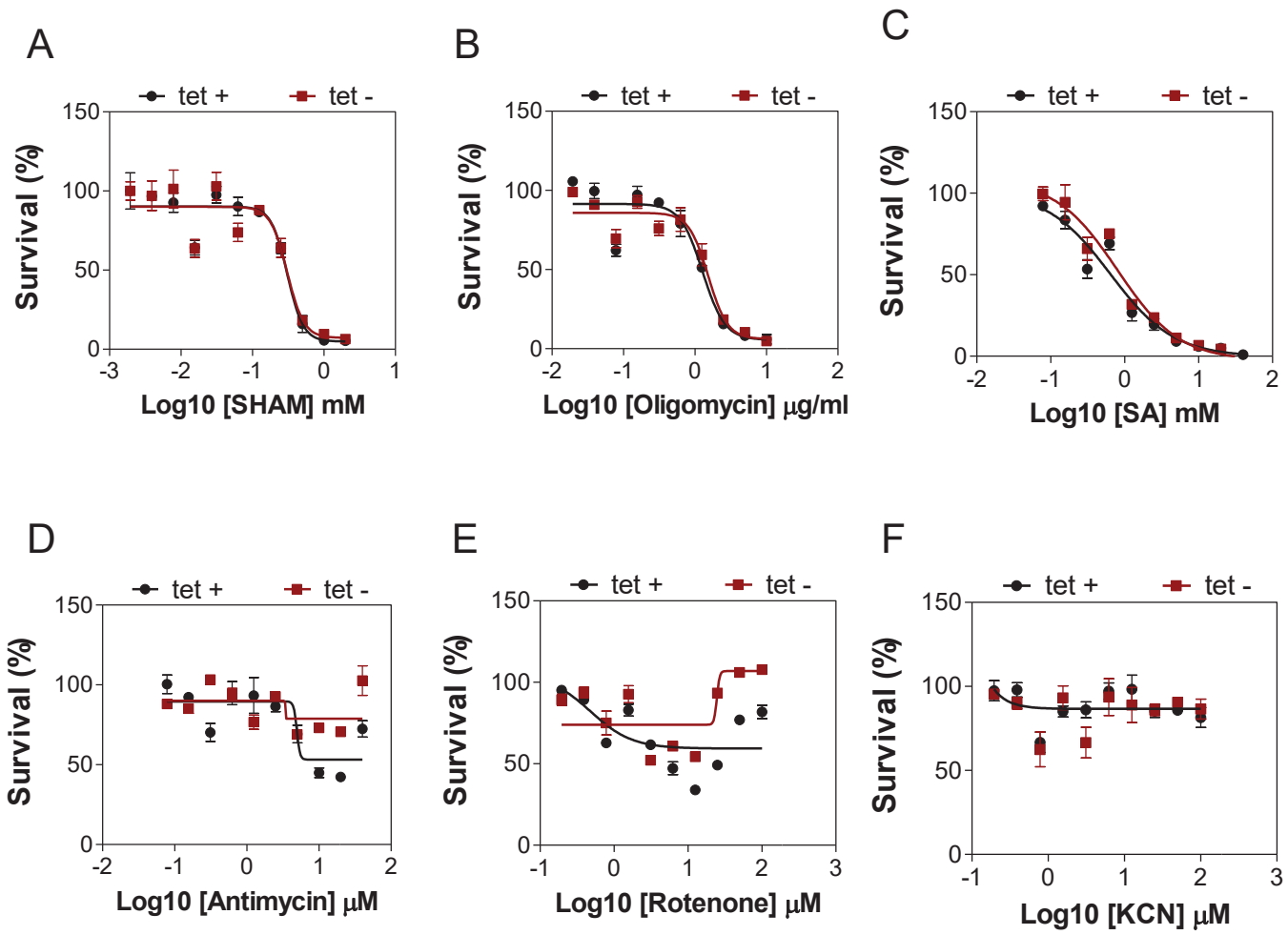

Figure S6. Viability assay of *T. brucei* IPMK CN in presence of oxidative phosphorylation inhibitors. A-F) *T. brucei* IPMK CN was growth in presence or absence of tet (0.5 µg/ml) for 36h in presence of various concentration of A) Salicylhydroxamic acid (SHAM, 2 mM – 0.04 mM); B) Oligomycin (10 – 0.02 µg/ml); C) Sodium Azide (SA, 40 – 0.08 µM); D) Antimycin (40 – 0.08 µM); E) Rotenone (100 – 0.2 µM); and F) potassium cyanide (KCN, 100 – 0.2 µM). Viability was measured with AlamarBlue. Data are represented as mean ± SEM.

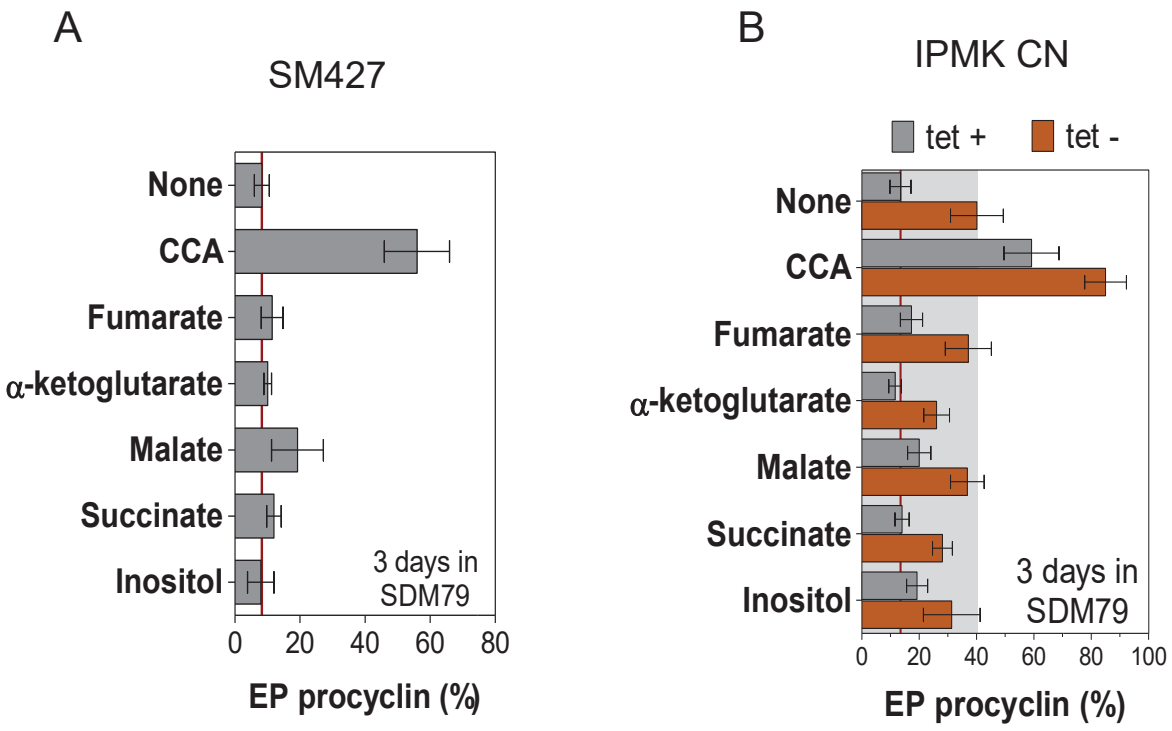

Figure S7. *T. brucei* EP procyclin expression in presence of TCA cycle intermediates and myo-inositol. A-B) Flow cytometry quantification of EP procyclin expression in *T. brucei* SM427 (A) or IPMK CNs (B) after 3 days culture in SDM-79 at 27°C in presence or absence of TCA cycle intermediates (10 mM, except CCA which was used at 6 mM) or myo-inositol (10 mM). IPMK was knocked down for 36h in HMI-9 at 37°C before transfer to SDM-79 at 27°C. Tet, 0.5 µg/ml. The red line in A indicates % of cells expressing EP procyclin without addition TCA or myo-inositol metabolites (None); whereas the gray shade in B indicates % of cells that express EP procyclins after IPMK knockdown in absence of TCA or myo-inositol metabolites (None). Data are represented as mean ± SEM.

Table S1. Antibodies used in this work.

Antibodies	Provider	Dilutions	Notes
pAb α-PGK (1789G) (3)	Dr. Marilyn Parsons (CIDR)	1:1,000 (IF) 1:10,000 (WB)	Recognizes PGKA, PGKB and PGKC
α-gGAPDH/Aldolase (2481D) (3)	Dr. Marilyn Parsons (CIDR)	1:1,000 (IF) 1:10,000 (WB)	Recognizes glycosomal GAPDH and Aldolase
mAbs α-procyclin (clone tbrp1/247)	Cedarlane laboratories	1:500 (IF) 1:1,000 (WB)	Recognizes EP procyclins
pAbs α-Pex14 (4)	Dr. Marilyn Parsons (CIDR)	1:5,000 (WB)	NA
pAbs α-AOX	Dr. Ken Stuart (CIDR)	1:1,000 (WB)	NA
mAbs α-VSGs (clones 3E1212-1, 12E29-9, TrypIII1A8$_4$, 5F118-1, 4F6$_{10\text{-}1}$, IST9368$_1$) (5)	Dr. Ken Stuart (CIDR)	1:1,000 (WB)	Mix of various α-VSG mAbs in equal proportions. Cross react with VSG2 (also known as VSG221). SM427 and IPMK CN (tet+) express VSG2.
mAbs α-HSP70 (78) (6)	Dr. Ken Stuart (CIDR)	1:1,000 (WB)	NA
pAb α-PAD1 (7)	Dr. Marilyn Parsons (CIDR)	1:50 (FC)	Antibody produced by Dr. Keith Matthews laboratory at the University of Edinburgh), aliquot received from Dr. Marilyn Parsons (CIDR).
goat α-rabbit IgG (H+L) Alexa Fluor 488®	Molecular Probes	1:1,000 (FC)	Secondary antibodies
goat α-rabbit IgG (H+L) Alexa Fluor 568®	Molecular Probes	1:1,000 (FC)	Secondary antibodies
goat α-mouse IgG (H+L) Alexa Fluor 488®	Molecular Probes	1:1,000 (FC)	Secondary antibodies
goat α-mouse IgG (H+L)-HRP	Bio-Rad	1:5,000 (WB)	Secondary antibodies
goat α-rabbit IgG (H+L)-HRP	Bio-Rad	1:10,000 (WB)	Secondary antibodies

Notes: pAb, polyclonal antibodies; mAb, monoclonal antibodies; NA, not applicable; IF, immunofluorescence; WB, Western blotting; FC, flow cytometry; CIDR, Center for Infectious Disease Research. For IF, antibodies were diluted in PBS 3% BSA; for FC in 5% FBS; and for WB in 5% low fat powdered milk.

Table S2. Primers used in this work.

Gene ID	Primer ID	Forward sequence	Reverse sequence	Use
Tb427.01.5160	BF_set_01	TGACGACGCAGCTCATCTTT	CCGCACAAATCCTCTCCACT	Gene expression
Tb427.08.3730	BF_set_02	GCGTTTTCATAGCGCGCTTA	CATTCCCAATTCAGCCGACG	Gene expression
Tb427.10.2440	BF_set_03	TTTGCGGTATTTGGCTTGGC	GCGTACCATGACCCGAGTAG	Gene expression
Tb427tmp.211.3955	BF_set_04	ACATGCAGACTCGCTTCCAA	TGTTTGAGCCGCTTCCTCAT	Gene expression
Tb427.02.3270	BF_set_05	GAAGCGCTATTGACTGCAGC	GGCTTCACATTCTCCCAGCT	Gene expression
Tb427.08.6720	BF_set_06	TGACAATGTCTGGGATGGGC	AATGAAAGCAGCAGCAACCG	Gene expression
Tb427.06.170	BF_set_07	GCTCAACAGGTTGCTGGTTG	ATTCCAAGCATCCACCCCTG	Gene expression
Tb427.08.6710	BF_set_08	CCACCCGACATGAAACCAGA	CCCAGCTGCACCAAGAGTAA	Gene expression
Tb427.02.2490	BF_set_09	GGACTGGAACACAAACGCAC	TTGGTGATGGGATGGAGCAC	Gene expression
Tb427.06.390	BF_set_10	CTTGGTGGTGTTGCACTTCG	ATTCGCGATCCAGCCGTAAT	Gene expression
Tb427.06.300	BF_set_11	GATTCAGCGCTTCCAGCATG	GCGTTGTCGTCCTCAAGGTA	Gene expression
Tb427.02.3320	BF_set_12	GAAGTCCACGGGCTTGTACA	GCTGCAGTCAATAGCGCTTC	Gene expression
Tb427.06.310	BF_set_13	CATATTGCAACGCGGCATGA	CTGTGACCCTGTGAGACGAC	Gene expression
Tb427.03.1490	BF_set_14	TCTCAATGCGACAGCCTTGT	ACTCCCCACAGTTGCACAAA	Gene expression
Tb427.06.280	BF_set_15	GATTCAGCGCTTCCAGCATG	AGAGAACTTCTCCCGCAAGC	Gene expression
Tb427.02.3310	BF_set_16	CCGCATATGCTGTGAACACG	ATTTTCGCGCCCTTTTTCCC	Gene expression
Tb427.10.8490	BF_set_17	AGGTACAAGTCGTCGGAGGA	CCATAAGAACCCACGGCCTT	Gene expression
Tb427.06.770	BF_set_18	GGTTGCGAGATTTCATCGCC	AAGCCCAGGAACGTTAGTGG	Gene expression
Tb427.06.200	BF_set_19	GTTTGGTGTGCCGCTTGATT	CAACCACAGCAACGTCAGTG	Gene expression
Tb427.03.4080	BF_set_20	TCGATTACGCTGGTCCGATG	TTTCCATCGAAGGTGAGGCC	Gene expression
Tb427.02.3300	BF_set_21	CCGCATATGCTGTGAACACG	ATTTTCGCGCCCTTTTTCCC	Gene expression
Tb427.01.5120	BF_set_22	TTGGAGCGTGACAGTGATCC	CCTTCTTTTAGCGGGCAGGA	Gene expression
Tb427tmp.02.5540	BF_set_23	GGGTGTGCACAGATGAGGAA	AAACCCCAATGCCGAGATGT	Gene expression
Tb427.06.290	BF_set_24	TTGGCAAAGGTCAACTGGGT	CACGACACCAAACACAGCAG	Gene expression
Tb427.02.3610	PF_set_01	ATGACGCCGTTATGCAATGC	GCGGCAAGTTTCTTTGCAGA	Gene expression
Tb427.08.2470	PF_set_02	ATGCCCATGCTTCAACCGTA	ACTGCTGCGGGTACTTTCTC	Gene expression
Tb427.07.7090	PF_set_03	AGAGGGATCCGCAACAGTTG	CGAAGTGTACTGAAGCGGGT	Gene expression
Tb427tmp.02.3860	PF_set_04	ACGGTGCTGAATGTGGTAGG	CCCAAAGACTGGTCCCCATC	Gene expression
Tb427.01.2260	PF_set_05	GGAAAGGCAAAGTTGGAGCG	GCGCCTGTCTCCATCAGTAA	Gene expression
Tb427.07.2700	PF_set_06	TCCCAACTCGAGGATGGGAT	ATTGTCTTCCATGGGCCCTG	Gene expression
Tb427.08.7600	PF_set_07	GCACTGCATTGCCGTTGTTA	GGCGGGAAATAGGAAACCCA	Gene expression
Tb427.10.2560	PF_set_08	TGAAAGCTAAAGGAGGGGCG	TACCCTTGTCACCACGAAGC	Gene expression
Tb427.07.2710	PF_set_09	TTCTTGTGCGTCCTTTTGCG	ACGAACGGTCCCTTGAACTC	Gene expression
Tb427.10.7180	PF_set_10	ACGGGACTGACGGAATGTTC	CACTGACGCTCGATCCTCTC	Gene expression

Tb427.08.7640	PF_set_11	GACCCTGTGAACTACCCAGC	GGCAACCTCTCTGAAGTCCC	Gene expression
Tb427.08.7670	PF_set_12	AGAAGTCAGGCAACCGACTG	ATGAAACTGACGGCGATGGT	Gene expression
Tb427.08.7630	PF_set_13	CCCAGCAGTTATGGTCGGTT	GGCAACCTCTCTGAAGTCCC	Gene expression
Tb427.04.5240	PF_set_14	CGTCTAATGTTGCTGCCGTG	ATGTCTGTCGCTGCAAGTCA	Gene expression
Tb427.08.7610	PF_set_15	GACCCTGTGAACTACCCAGC	GGCAACCTCTCTGAAGTCCC	Gene expression
Tb427.08.7650	PF_set_16	AGAAGTCAGGCAACCGACTG	GGCGATGACAAGGTACACCA	Gene expression
Tb427.08.7620	PF_set_17	CCCAGCAGTTATGGTCGGTT	GGCAACCTCTCTGAAGTCCC	Gene expression
Tb427tmp.02.4520	PF_set_18	GAACAAGCGTTTCGGCACTT	CATAAGCACCCTCAACCCGT	Gene expression
Tb427.04.5260	PF_set_19	CGGGCAAGTTTGGTGTGAAG	CGGCTTCCTGTATGTGGTGT	Gene expression
Tb427.08.8290	PF_set_20	TATCTGTTGCGGCACTGGTT	AACACGTTGCCATGTGAAGC	Gene expression
Tb427.01.2230	PF_set_21	ACCTTTAGCGAGGACTGCAG	CAGTTGCCACACTTTCACCG	Gene expression
Tb427.08.8300	PF_set_22	ATGCCCATGACCACGGAAAT	CCGCCTGGAGGTAAAACAGT	Gene expression
Tb427.04.5250	PF_set_23	CGGGCAAGTTTGGTGTGAAG	CGGCTTCCTGTATGTGGTGT	Gene expression
Tb427tmp.03.0570	PF_set_24	TGTTTCTGAGGCAGGCGAAT	CGTTATGCAACATCCGCCTG	Gene expression
Tb427.03.2930	RBP6	ATGTTCTACCCCAACAGCCC	CTGCCCGTATGGAATTTGCG	Gene expression
Tb427tmp.03.0580	UBP2	CGGCCCAATTGAGTCGGTTA	TAACCGCGACTTTGACGAGT	Gene expression
Tb427.10.7470	RBP15	TCGAGAGCGAATGTCCGATG	CACAGCTTTGTGGGAATCCC	Gene expression
Tb427.10.8300	RBP14A	CAGATTGGTTATAATGGTAA	GTCTAAGAAAAAGCACAGTT	Gene expression
Tb427.10.12090	RBP7A	GTACGGAGGAGGCAGATTGT	CGCGCTCCATATTGTAGCG	Gene expression
Tb427.10.12100	RBP7B	GGTGATGTGCTGCACGTTAA	CGTGTTGCAACGGTTGTGAA	Gene expression
Tb427.05.3750	PPCT1	CACCACACAGACGGTTGACA	GTATAAGTCGAGACGGCCGG	Gene expression
Tb427.08.2780	RBP10	AAGGACTTAACGGCCGAGTG	CGTCTGCTATTCGTGCTTGC	Gene expression
Tb427.08.4830	RBP24	GGAACCTGAGTTTCTGCCGA	ATCGACGTCCCCTGCAATTT	Gene expression
Tb427tmp.01.8310	RBP37	GGCATGTCGTCTGTTGAGGA	CAATGTGGCGCCATTGAGAC	Gene expression
Tb427.01.720	PGKA	GTTTTTCTGATGTCGTGGGAGT	TGTACTTCTCAAACAACGTTCACA	Gene expression
Tb427.01.710	PGKB	GTCACTAAAAGAGAGGAAGA	AATATTACCATCATCCAGAG	Gene expression
Tb427.01.700	PGKC	CGGTTGTGTCGTATGCCTCT	AAAGAGAGCTCCACCGGTTA	Gene expression
Tb927.11.10190	Telomerase reverse transcriptase	GAGCGTGTGACTTCCGAAGG	AGGAACTGTCACGGAGTTTGC	Gene expression
Tb927.10.5330	18S	CGGAATGGCACCACAAGAC	TGGTAAAGTTCCCCGTGTTGA	Gene expression
Tb927.1.2330	β-tubulin	TTCCGCACCCTGAAACTGA	TGACGCCGGACACAACAG	Gene expression
Tb927.7.3490	qPCR_67	GTTAAAGGAACAGGGTATT	GTTAAAGGAACAGGGTATT	Gene expression
Tb927.8.7170	qPCR_63	TTTTTACAGCACTGTTGAT	GTCATTCCACACGTATGATA	Gene expression
Tb927.7.3490	qPCR_67_5_UTR	CCACTGTGGAAATAAGG	CTACAAAAAGTATACTTGTTCC	Genotyping
Tb927.7.3490	qPCR_67_3_UTR	CCAAATCACTTCGGAGT	CCGTTCACTTCCATGAA	Genotyping
Tb927.8.7170	qPCR_63_5_UTR	CTTTTGTAAAGTGTTAATCTGT	CGAAGCTGAATCAGATC	Genotyping
Tb927.8.7170	qPCR_63_3_UTR	TCAAAACCCTTCCTGAC	CCATACGTTCATATGACTAA	Genotyping
Tb427.03.2930	RBP6_HindIII and RBP6_BamHI	CCCAAGCTTATGTTCTACCCCAACAGCC	CCCGGATCCACCAGCGGCTCCG	Cloning in pLEW100-V5

Tb427.10.12090	RBP7A_HindIII and RBP7A_BamHI	CCCAAGCTTATGCCACCGCGGGCTC	CCCGGATCCCCGTTGAACACGTTGTGGT	Cloning in pLEW100-V5
Tb427.10.12100	RBP7B_HindIII and RBP7B_BamHI	CCCAAGCTTATGCCACCGCGGGCTC	CCCGGATCCACGTTGCACACGTTGTGGTGG	Cloning in pLEW100-V5
Tb427.05.3750	PPCT1_BSAI_(HindIII) and PPCT1_BSAI_(BamHI)	CCCGGTCTCAAGCTTATGCCAAGTTCGTCGTTGT	CCCGGTCTCGGATCCTTGGCGCGCCCTCTTG	Cloning in pLEW100-V5
Tb927.8.7170	5' F1 and 3' R1	CTCAGGTGCCACCTCCAA	GGTACAACTCTGTCACACCCTTAG	Knockout construct
Tb927.8.7170	3' HYG - F and 5' HYG - R	GTCCGAGGGCAAAGGAATAGTCGCTACAGGAAATCAATAAATCG	GGTGAGTTCAGGCTTTTTCATAAGCTGAATGATATAAGACAGATTAACACTTTAC	Knockout construct
Tb927.8.7170	3' BSR - F and 5' BSR - R	TGGTTATGTGTGGGAGGGCTAATCGCTACAGGAAATCAATAAATCG	TTGAGACAAAGGCTTGGCCATAAGCTGAATGATATAAGACAGATTAACACTTTAC	Knockout construct
Tb927.8.7170	5' F2 and 3' R2	TACCACATTTTTGATCTGGTTGC	GTGCAGGTCTTTTCCTTCACTCT	Knockout construct
Tb927.7.3490	5' F1 and 3' R1	TACATATGTACATGTGTGCAAGTGTGTAT	GCGCGATACGCACAAAA	Knockout construct
Tb927.7.3490	3' HYG - F and 5' HYG - R	GTCCGAGGGCAAAGGAATAGATGTTGCTCGAAATGAACTGG	GGTGAGTTCAGGCTTTTTCATTTTTTCAGCCCTATTCAAAAACG	Knockout construct
Tb927.7.3490	3' BSR - F and 5' BSR - R	TGGTTATGTGTGGGAGGGCTAAATGTTGCTCGAAATGAACTGG	TTGAGACAAAGGCTTGGCCATTTTTTCAGCCCTATTCAAAAACG	Knockout construct
Tb927.7.3490	5' F2 and 3' R2	CGTTTTTCTTCCCTTTTAACCG	TGCAATAATGAGGCAGATAGAAGTTT	Knockout construct
Tb927.8.7170	PCR_fusion_63	TTACACCAAAAAGTAAAATTCACAAGCTTATGCCTCAGGTGGATCTCG	AAAGACACGAGACAAGGGAAAAGCTACTGCCATCCCAAACGC	Cloning in pLEW100
Tb927.7.3490	PCR_fusion_67	TTACACCAAAAAGTAAAATTCACAAGCTTATGTACGTGGAGAAGAGCC	GAGAGGGTTAGGGATAGGCTTACCTTACTTGTATAATCCCCGCACGAAATT	Cloning in pLEW100
Tb927.9.12470	IPMK	CCCGGTCTCAAGCTTATGTTAAATATTTGCCAAAACTTGTCTTC	CCCGGTCTCGGATCCTGAAAGAAGAAAAATAATTTTTTCCA	Cloning in pET29a
Tb927.9.12470	IPMK mutation D142A	TTGTGTGCTTGCTATCAAACTTG	GGTTTATGAAATGTCGCG	Cloning in pHD1344-V5 *tub*
Tb927.9.12470	IPMK mutation K164W	GCGCATACATTGGAGGCAGCTTC	TCCACCTTGTCGGGTAAT	Cloning in pHD1344-V5 *tub*
Tb927.9.12470	IPMK mutation D142A/K144A	GCTTGCTATCGCACTTGGATATGTG	ACACAAGGTTTATGAAATGTC	Cloning in pHD1344-V5 *tub*

Table S3. RNA sequencing analysis of *T. brucei* after 24h and 36h of IPMK knockdown.

	24h tet +		24h tet -		36h tet +		36h tet -	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Total reads (millions)	100,82	9.32	63.85	7.92	6.93	9.41	5.56	98.33
Read counts mapped to genes (millions)	47.50	5.47	39.48	4.72	3.48	3.48	2.78	61.82
Average read counts/gene	5,184	608	4,308	523	414	401	324	6755
Number of mapped genes	9,164	9,004	9,165	9,025	8,382	8,713	8,589	9,153
Read length (bp)	50	50	50	50	36	50	36	50
Paired (P) or unpaired (NP)	NP	NP	NP	NP	P	NP	P	NP
Pearson's coefficient of correlation Read counts (Rep 1 vs Rep 2)	0.99		0.99		0.98		0.98	
*Pearson's coefficient of correlation DE (log 2FC) (Rep 1 vs DE Rep 2)	0.883				0.70			

Notes: DE, differential expression (comparing tet – vs. tet +); Pearson's coefficient of correlation comparing log 2 fold-changes between replicates. * Note that for the purpose of analyzing correlation between replicates only, DE of each replicate was analyzed separately.

Supporting Material and Methods

1. RNAseq analysis and gene set enrichment analysis (GSEA)

RNAseq libraries of poly-A enriched RNAs was performed as previously described (8). Libraries were sequenced at the Department of Genome Sciences, University of Washington (Table S7). Two biological replicates were performed for each RNAseq experiment. Reads alignment and gene ID mapping were done using Bowtie2 and HTSeq against *T. brucei* 427 genome tritrypDB version 9, respectively, read counts were obtained with Get_ReadCount.py (in house software) and differential gene expression using edgeR (9) using a likelihood ratio test (10). Libraries were filtered for genes with low read using a minimum of one count per million (CPM) per gene, *i.e.* 5 counts per gene in the smallest library, and counts were required to be present in at least two libraries of the same biological replicate group for a gene to be considered for analysis. Genes were considered differentially expressed when fold-changes ≥ 2 and *p*-values ≤ 0.05. For GSEA the phenoTest package in R was used (11, 12). Gene sets were constructed with genes that are differentially expressed in BF slender, intermediate and stumpy forms and PF (13), and include genes which are upregulated (72 genes) or downregulated (254 genes) in BF stumpy compared to BF slender; and upregulated (130 genes) or downregulated (178 genes) BF intermediate compared to BF slender. It also include sets of genes which are upregulated in BF compared to PF (157 genes), or PF compared to BF (70 genes). Gene sets were analyzed using 1,000 permutation tests by comparing gene sets against the RNAseq data set IPMK knockdown at 36h. False discovery rates (q) were calculated by Benjamini–Hochberg procedure and significance indicated by *q*-value <0.05. Gene sets were represented using barcode plot using Limma package in R (14).

2. Differentiation and flow cytometry analysis

IPMK knockdown and expression of PAD1: *T. brucei* IPMK CN were grown at late log phase (~1.5x10^6 parasites/ml) with or without tet (0.5 µg/ml) for 24h and 36h in 40 ml HMI-9 medium at 37°C. Afterwards, 10 ml aliquots were fixed in 1% paraformaldehyde (PFA, vol/vol) in PBS for 30 minutes at RT. Fixed cells were centrifuged for 5 minutes at 4,000 rpm at RT and blocked in PBS 10% FBS (vol/vol) for 1h at RT. Cells were washed by centrifugation as above and incubated in PBS 5% FBS (vol/vol) with pAb α-PAD1 with gentle shacking (see Table S6 for antibody dilutions) for 2h at RT, then washed three times with 1 mL of PBS by centrifugations, as described above, and then incubated with goat α-rabbit IgG (H+L) Alexa Fluor 568® (Molecular Probes, Table S6) for 1h at RT with gentle shacking. Cells were washed three times in PBS by centrifugation and analyzed by flow cytometry using a BD™ LSR II flow cytometer (BD Biosciences) and FlowJo software (Flowjo, LLC).

IPMK overexpression and EP procyclin analysis: *T. brucei* BF that express tet-reguatable C-terminally V5-tagged IPMK were grown at late log phase (~1.5x10^6 parasites/ml) without tet in HMI-9 a 37^0C for 24h. Afterwards, cells were centrifuged for 5 min at 4,000 rpm at RT and transferred to 24-well plates at 2.0x10^7 parasites/ml without or with tet (1 µg/ml) and cultured in SDM-79 at 27^0C for 3 days in presence of various concentrations of CCA (0.16, 0.5, 1, 3 and 6 mM). Afterwards, cells were fixed in 1% PFA (vol/vol) in PBS for 30 minutes at RT. Cells were washed by centrifugation as above and incubated in PBS 5% FBS (vol/vol) with mAb α-EP procyclin (clone tbrp1/247) with gentle shacking (see Table S6 for antibody dilutions) for 2h at RT. Cells were then washed three times with 1 mL of PBS by centrifugations and then incubated with goat α-mouse IgG (H+L) Alexa Fluor 488® (Molecular Probes, Table S6) for 1h at RT with gentle shacking. Cells were washed three times in PBS by centrifugation and analyzed by flow cytometry as described above.

Differentiation analysis of T. brucei IPMK CN that exclusively express T. cruzi or L. major IPMK: *T. brucei* IPMK CN or which contains a copy of *L.major* IPMK or *T. cruzi* IPMK introduced in the tubulin locus for constitutive expression (15) were grown at late log phase (~1.5x10^6 parasites/ml) with tet (0.5 µg/ml) for 36h in 40 ml HMI-9 medium at 37°C. Afterwards, cells were washed twice in HMI-9 without tet and 2 mL aliquots were transferred to 24-well plates at 2.0x10^7 parasites/mL without or with tet (1 µg/ml) and cultured in SDM-79 at 27^0C for 3 days. Afterwards, cells were fixed in 1% PFA (vol/vol) in PBS for 30 minutes at RT. Cells were stained with α-EP procyclin antibodies (as described above) and analyzed by flow cytometry using a BD™ LSR II flow cytometer (BD Biosciences) and FlowJo software (Flowjo, LLC).

Differentiation of T. brucei IPMK CN in presence of TCA cycle or myo-inositol metabolites: *T. brucei* IPMK CN were grown at late log phase (~1.5x10^6 parasites/ml) with or without tet (0.5 µg/ml) for 36h in 40 ml HMI-9 medium at 37°C. Afterwards, cells were washed twice in HMI-9 without tet and aliquots of 2.0x10^7 parasites/mL were transferred to 24-well plates in SDM-79 and cultured for 3 days at 27°C in absence or presence of CCA (6 mM), malate (10 mM), α-ketoglutarate (10mM), succinate (10 mM), or myo-inositol (10 mM). After day 3, cells were fixed in 1% PFA and aliquots of 1 ml of cells were collected for EP procyclin expression analysis by flow cytometry as described above.

3. Growth curve analysis

Cumulative growth curve analyses of *T. brucei* SYJ 2 or IP1Pase CNs were performed as previously described (16). Briefly, *T. brucei* BF were grown in HMI-9 at 37^0C with 5% CO_2 I n presence or absence of tet (0.5 µg/mL) and counted daily using a cell counter (Beckman) and diluted daily to 5.0 x 10^4 parasites/ml in new medium, and the procedure was repeated for 7 consecutive days.

4. Viability assays

Viability assays were performed as previously described (17). Briefly, compound stocks were prepared at a 10 mM – 1M concentration in dimethyl sulfoxide or Ethanol. *T. brucei* BF (100 µL at 2.0 x 10^4 parasites/ml) were plated in 96-well plates and mixed with 100 µL of compounds at 2-fold serial dilutions, *i.e.* SHAM (100 mM – 0.2 mM), Oligomycin (10 – 0.02 µg/ml), Sodium Azide (40 – 0.08 µM), Antimycin (40 – 0.08 µM), Rotenone (100 – 0.2 µM), and potassium cyanide (100 – 0.2 µM), all diluted in HMI-9 medium with 10% FBS. Parasites not treated with compounds were also plated as controls. After 36h of incubation at 37 °C and 5% CO2, 20µL of alamarBlue (Invitrogen) was added, and the assays were developed for 4h. Fluorescence measurements were obtained using a SpectraMax M2 microplate reader (Molecular Devices) with excitation at 544 nm and emission at 590 nm (590-nm cutoff). Data were analyzed using GraphPad Prism for Windows.

Supporting References

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