Global quantitative SILAC phosphoproteomics reveals differential phosphorylation is widespread between the procyclic and bloodstream form lifecycle stages of *Trypanosoma brucei*

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Supplementary Information:

Table S1. Bloodstream form cell dimensions measured by light scattering.

	HMI9-T	HMI11-SILAC
Mean diameter / μM	3.94	4.10
Mean volume / μM^3	36	40

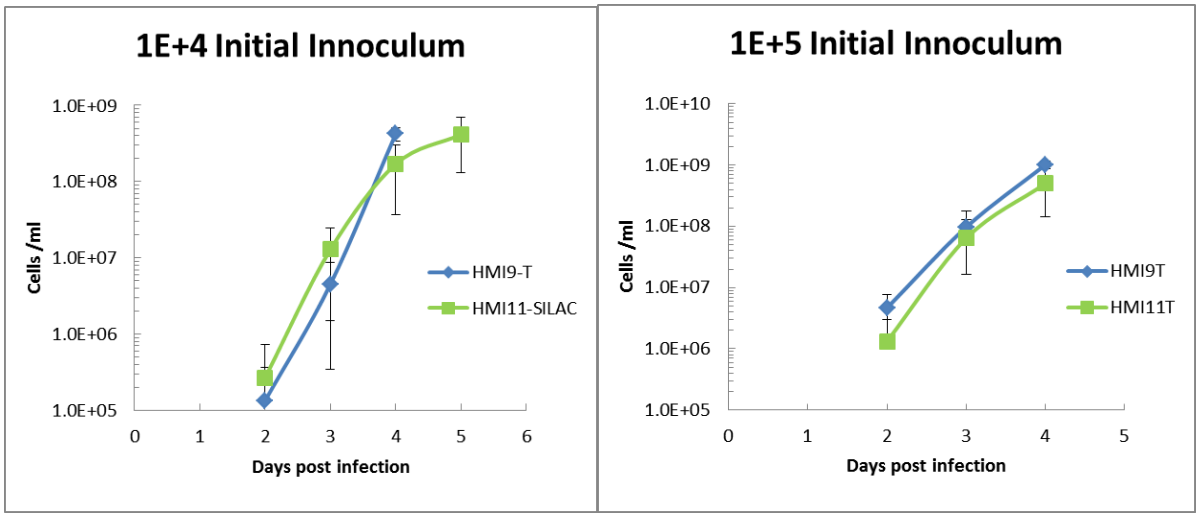


Figure S1. Rodent infectivity of bloodstream form *T. brucei* cultured in HMI9-T and HI11-SILAC media. Bloodstream form cells grown HMI9-T or HMI11-SILAC + R_0K_0 for >14 days prior to inoculation of female Balbc mice (three per condition) by inter peritoneal injection of 1×10^3, 1×10^4 or 1×10^5 cells in the same media. Daily tail bleeds were used to monitor the number of parasites in the bloodstream using a heamocytometer, and animals were euthanized when the parasite burden exceeded 5×10^8 cells/ml prior to overt distress.

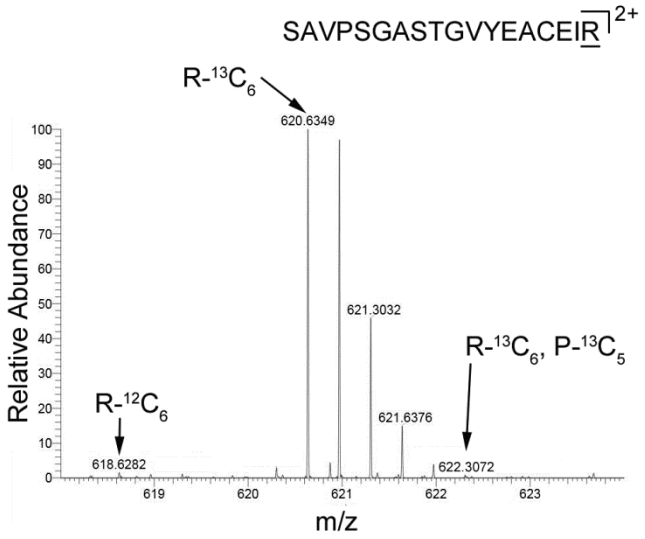
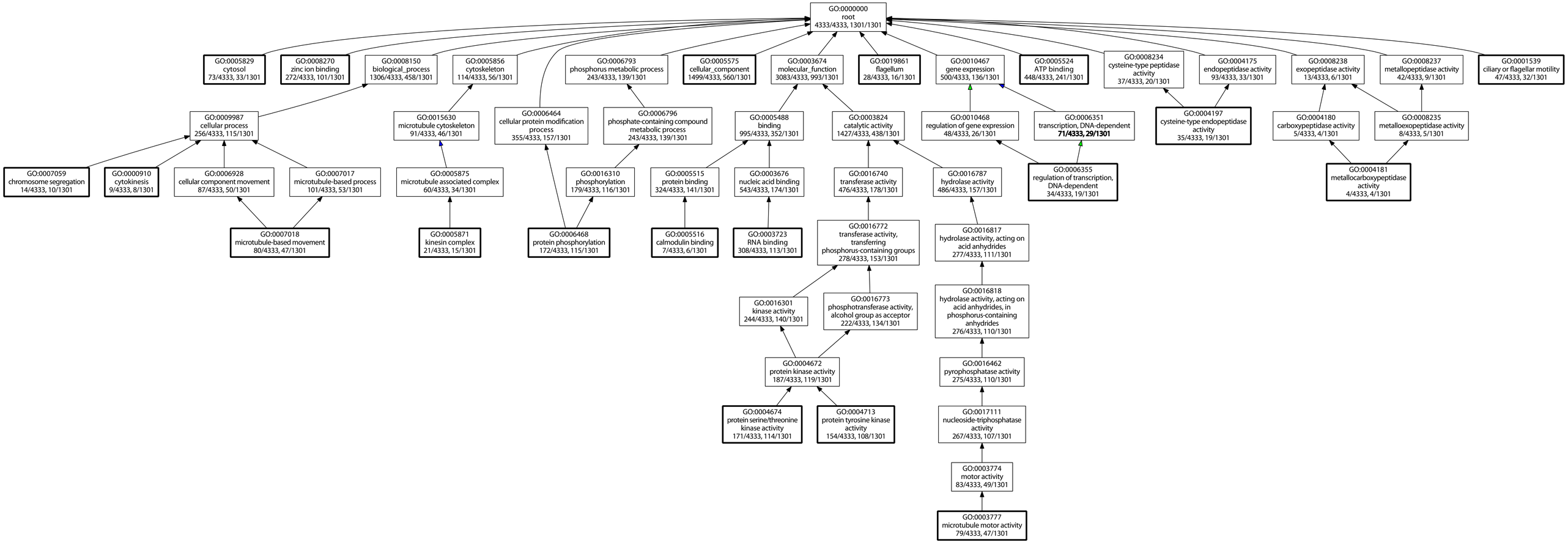


Figure S2. Efficiency of isotope incorporation in bloodstream form *T. brucei*. Mass spectrum of a doubly charged peptide from enolase (Tb927.10.2890) obtained after three days growth in HMI11-SILAC+R_6K_4.

Figure S3 (overleaf). Gene Ontology term enrichment for all phosphoproteins. Term-for-term enrichment, $P < 0.01$, uncorrected due to sample size. Each box contains the following information: GO term ID and description, Number of terms in population / Total number of terms in population, Number of terms in set / Total number of terms in set.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root
4333/4333, 1301/1301
GO:0005829
cytosol
73/4333, 33/1301
GO:0008270
zinc ion binding
272/4333, 101/1301
GO:0008150
biological_process
1306/4333, 458/1301
GO:0005856
cytoskeleton
114/4333, 56/1301
GO:0006793
phosphorus metabolic process
243/4333, 139/1301
GO:0005575
cellular_component
1499/4333, 560/1301
GO:0003674
molecular_function
3083/4333, 993/1301
GO:0019861
flagellum
28/4333, 16/1301
GO:0010467
gene expression
500/4333, 136/1301
GO:0005524
ATP binding
448/4333, 241/1301
GO:0008234
cysteine-type peptidase activity
37/4333, 20/1301
GO:0004175
endopeptidase activity
93/4333, 33/1301
GO:0008238
exopeptidase activity
13/4333, 6/1301
GO:0008237
metallopeptidase activity
42/4333, 9/1301
GO:0001539
ciliary or flagellar motility
47/4333, 32/1301
GO:0009987
cellular process
256/4333, 115/1301
GO:0015630
microtubule cytoskeleton
91/4333, 46/1301
GO:0006464
cellular protein modification process
355/4333, 157/1301
GO:0006796
phosphate-containing compound metabolic process
243/4333, 139/1301
GO:0005488
binding
995/4333, 352/1301
GO:0003824
catalytic activity
1427/4333, 438/1301
GO:0010468
regulation of gene expression
48/4333, 26/1301
GO:0006351
transcription, DNA-dependent
71/4333, 29/1301
GO:0004197
cysteine-type endopeptidase activity
35/4333, 19/1301
GO:0004180
carboxypeptidase activity
5/4333, 4/1301
GO:0008235
metalloexopeptidase activity
8/4333, 5/1301
GO:0007059
chromosome segregation
14/4333, 10/1301
GO:0000910
cytokinesis
9/4333, 8/1301
GO:0006928
cellular component movement
87/4333, 50/1301
GO:0007017
microtubule-based process
101/4333, 53/1301
GO:0005875
microtubule associated complex
60/4333, 34/1301
GO:0016310
phosphorylation
179/4333, 116/1301
GO:0005515
protein binding
324/4333, 141/1301
GO:0003676
nucleic acid binding
543/4333, 174/1301
GO:0016740
transferase activity
476/4333, 178/1301
GO:0016787
hydrolase activity
486/4333, 157/1301
GO:0006355
regulation of transcription, DNA-dependent
34/4333, 19/1301
GO:0004181
metallocarboxypeptidase activity
4/4333, 4/1301
GO:0007018
microtubule-based movement
80/4333, 47/1301
GO:0005871
kinesin complex
21/4333, 15/1301
GO:0006468
protein phosphorylation
172/4333, 115/1301
GO:0005516
calmodulin binding
7/4333, 6/1301
GO:0003723
RNA binding
308/4333, 113/1301
GO:0016772
transferase activity, transferring phosphorus-containing groups
278/4333, 153/1301
GO:0016817
hydrolase activity, acting on acid anhydrides
277/4333, 111/1301
GO:0016301
kinase activity
244/4333, 140/1301
GO:0016773
phosphotransferase activity, alcohol group as acceptor
222/4333, 134/1301
GO:0016818
hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides
276/4333, 110/1301
GO:0004672
protein kinase activity
187/4333, 119/1301
GO:0016462
pyrophosphatase activity
275/4333, 110/1301
GO:0004674
protein serine/threonine kinase activity
171/4333, 114/1301
GO:0004713
protein tyrosine kinase activity
154/4333, 108/1301
GO:0017111
nucleoside-triphosphatase activity
267/4333, 107/1301
GO:0003774
motor activity
83/4333, 49/1301
GO:0003777
microtubule motor activity
79/4333, 47/1301

Supplementary data supplied as separate files:

Assignment of MS^2 spectra for protein kinase phosphorylation sites – pdfs contained in a zipped file.
Table S2. Protein groups including SILAC ratios
Table S3. Peptides including SILAC ratios
Table S4. Phosphorylation sites including SILAC ratios
Table S5. Phosphopeptides including SILAC ratios
Table S6. Manual validation of protein kinase of phosphorylation sites
Table S7. Phosphorylation sites > 10-fold up-regulated in bloodstream form
Table S8. Phosphorylation sites > 10-fold up-regulated in Procyclic form
Table S9. Phosphoproteins with phosphorylation sites differentially regulated between lifecycle Stages

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