

Table S3. Signal peptide (SP) and transmembrane domain (TMD) predictions of high confidence protein identifications (1% false discovery rate, protein probability ≥ 0.95) from *T. brucei* acidocalcisome datasets (ACCS1 and ACCS2). Predictions of TMD and SP for individual proteins were based on consensus of two or more algorithms. If the predicted number of TMD varied among the different predictions packages, we report the median number of TMD. If a protein group contained more than one protein hit (see Methods for explanation of protein grouping based on degenerate peptide fingerprints), the number of TMD and presence of SP were assigned if predicted in at least two members.

Protein Group	GeneID	Description	Group Consensus		TMHMM 2.1 # of TMD	HMMTOP 2.1 # of TMD	SignalIP 4.1 Signal Peptide?	Polyphobius # of TMD	Individual Consensus TM	Individual Consensus Signal Peptide?
			# of TMD	Signal Peptide?						
1	Tb927.6.3740	heat shock 70 kDa protein, mitochondrial precursor, putative								
	Tb927.6.3750	heat shock 70 kDa protein, mitochondrial precursor, putative								
	Tb927.6.3800	heat shock 70 kDa protein, mitochondrial precursor, putative								
2	Tb927.11.6280	pyruvate phosphate dikinase								
3	Tb927.1.2330	beta tubulin								
	Tb927.1.2350	beta tubulin								
	Tb927.1.2370	beta tubulin								
	Tb927.1.2390	beta tubulin								
4	Tb927.10.6400	chaperonin HSP60, mitochondrial precursor						1		
	Tb927.10.6510	chaperonin HSP60, mitochondrial precursor						1		
5	Tb927.3.1380	ATP synthase beta chain, mitochondrial precursor,ATP synthase F1, beta subunit						1		
6	Tb927.2.4210	glycosomal phosphoenolpyruvate carboxykinase								
7	Tb927.11.7460	glucose-regulated protein 78, putative,luminal binding protein 1 (BiP), putative						1		
	Tb927.11.7510	glucose-regulated protein 78, putative,luminal binding protein 1 (BiP), putative						1		
8	Tb927.10.5620	fructose-bisphosphate aldolase, glycosomal								
9	Tb927.1.2340	alpha tubulin								
	Tb927.1.2360	alpha tubulin								
	Tb927.1.2380	alpha tubulin								
	Tb927.1.2400	alpha tubulin								
10	Tb927.6.4280	glyceraldehyde 3-phosphate dehydrogenase, glycosomal								
	Tb927.6.4300	glyceraldehyde 3-phosphate dehydrogenase, glycosomal								
11	Tb927.8.3530	glycerol-3-phosphate dehydrogenase [NAD $_1$], glycosomal						1		
12	Tb927.11.11330	heat shock protein 70								
13	Tb927.11.9980	2-oxoglutarate dehydrogenase E1 component, putative								
14	Tb927.10.15410	glycosomal malate dehydrogenase						4		
15	Tb927.9.12570	glycerol kinase, glycosomal						1		
16	Tb927.5.930	NADH-dependent fumarate reductase								
17	Tb927.11.3980	mitochondrial processing peptidase alpha subunit, putative,metallo-peptidase, Clan ME, Family M16						1		
18	Tb927.2.4230	NUP-1 protein, putative								
19	Tb927.3.2230	succinyl-CoA synthetase alpha subunit, putative								
20	Tb927.5.1060	mitochondrial processing peptidase, beta subunit, putative,metallo-peptidase, Clan ME, Family M16								
21	Tb927.11.5520	triosephosphate isomerase						2		
22	Tb927.4.2070	antigenic protein, putative								
23	Tb927.11.11680	2-oxoglutarate dehydrogenase E2 component, putative						1		
24	Tb927.10.8030	hypothetical protein, conserved								
25	Tb927.10.7410	succinyl-CoA ligase [GDP-forming] beta-chain, putative-with=GeneDB:LmjF36.2950						1		
26	Tb927.9.5900	glutamate dehydrogenase								
27	Tb927.5.2930	hypothetical protein, conserved						1		
28	Tb927.5.2080	guanosine monophosphate reductase, putative						1		
29	Tb927.9.5320	nucleolar RNA binding protein, putative						1		
30	Tb927.8.2770	inositol 1,4,5-triphosphate receptor						5		
31	Tb927.10.3210	delta-1-pyrroline-5-carboxylate dehydrogenase, putative						5		
32	Tb927.11.1020	ribokinase, putative								
33	Tb927.11.2690	succinyl-coA:3-ketoacid-coenzyme A transferase, mitochondrial precursor, putative								
34	Tb927.11.1450	2-oxoglutarate dehydrogenase E1 component, putative								
35	Tb927.10.14820	mitochondrial carrier protein, putative,ADP/ATP translocase 1, putative						3		
	Tb927.10.14830	mitochondrial carrier protein, putative,ADP/ATP translocase 1, putative						3		
	Tb927.10.14840	mitochondrial carrier protein, putative,ADP/ATP translocase 1, putative						3		
36	Tb927.10.14550	ATP-dependent DEAD/H RNA helicase, putative								
37	Tb927.10.2090	elongation factor 1-alpha,EF-1-alpha								
	Tb927.10.2100	elongation factor 1-alpha,EF-1-alpha								
	Tb927.10.2110	elongation factor 1-alpha								
38	Tb927.7.7420	ATP synthase alpha chain, mitochondrial precursor,ATP synthase F1, alpha subunit						Y		
	Tb927.7.7430	ATP synthase alpha chain, mitochondrial precursor,ATP synthase F1, alpha subunit						1		
39	Tb927.10.2020	hexokinase						1		
40	Tb927.10.1510	NOT1						1		
41	Tb927.2.1560	cyclophilin-type peptidyl-prolyl cis-trans isomerase, putative								
	Tb927.2.1680	cyclophilin-type peptidyl-prolyl cis-trans isomerase, putative								
42	Tb927.3.3580	lipophosphoglycan biosynthetic protein, putative						1		
43	Tb927.3.1790	pyruvate dehydrogenase E1 beta subunit, putative								
44	Tb927.10.7570	dihydroxyacetone acetyltransferase E2 subunit, putative								
45	Tb927.5.3400	calcium-translocating P-type ATPase,calcium pump						10		
46	Tb927.8.3750	nucleolar protein, putative						10		
47	Tb927.1.4100	cytochrome oxidase subunit IV						1		
48	Tb927.8.2540	3-ketoacyl-CoA thiolase, putative								
49	Tb927.8.5640	hypothetical protein, conserved								
50	Tb927.10.1170	intraflagellar transport protein IFT172, putative								
51	Tb927.10.12700	pyruvate dehydrogenase E1 alpha subunit, putative								



476	Tb927.10.510	hypothetical protein, conserved			2							
477	Tb927.9.10520	hypothetical protein, conserved				Y						
478	Tb927.10.15710	mitochondrial carrier protein, putative					1					
479	Tb927.4.3840	nucleolar protein, putative						1				
480	Tb927.11.13890	hypothetical protein, conserved							Y			
481	Tb927.7.5790	protein disulfide isomerase, putative								Y		
482	Tb927.7.7260	kinesin, putative									Y	
483	Tb927.4.1280	hypothetical protein, conserved				1						
484	Tb927.3.2080	hypothetical protein, conserved					1					
485	Tb927.11.7520	hypothetical protein, conserved						1				
486	Tb927.5.3800	glutamine hydrolysing (not ammonia-dependent) carbamoyl phosphate synthase, putative										
487	Tb927.11.13310	hypothetical protein, conserved										
488	Tb927.7.7210	hypothetical protein, conserved										
489	Tb927.11.16750	hypothetical protein, conserved			1			1				
490	Tb927.10.830	adenylate kinase, putative						2				
491	Tb927.7.6990	hypothetical protein, conserved			1			1				
492	Tb927.11.5250	hypothetical protein, conserved			3			1				
493	Tb927.10.8980	hypothetical protein, conserved										
494	Tb927.11.12040	hypothetical protein, conserved										
495	Tb927.11.540	ABC transporter, putative										
496	Tb927.11.6430	hypothetical protein, conserved										
497	Tb927.1.3450	hypothetical protein, conserved										
498	Tb927.8.6640	hypothetical protein, conserved										
499	Tb927.11.610	hypothetical protein, conserved										
500	Tb927.8.1870	Golgi/lysosome glycoprotein 1										
501	Tb927.8.6050	hypothetical protein, conserved			1			1				
502	Tb927.6.2870	hypothetical protein, conserved			1			1				
503	Tb927.6.630	hypothetical protein, conserved			1			1				
504	Tb927.7.4290	hypothetical protein, conserved										
505	Tb927.5.4040	hypothetical protein, conserved										
506	Tb927.10.12960	ras-related protein rab-5, small GTPase, putative										
507	Tb927.10.2550	malate dehydrogenase-related										
508	Tb927.3.1820	hypothetical protein, conserved										
509	Tb927.6.3670	paraflagellar rod component, putative										
510	Tb927.5.1680	hypothetical protein, conserved										
511	Tb927.8.4450	RNA-binding protein, putative										
512	Tb927.6.4080	hypothetical protein, conserved										
513	Tb927.6.1870	eukaryotic translation initiation factor 4e, putative										
514	Tb927.11.6660	hypothetical protein, conserved										
515	Tb927.9.14420	cyclophilin-like protein, putative										
516	Tb927.10.9820	mitochondrial intermediate peptidase, putative, metallo-peptidase, Clan MA(E) Family M3			1			1				
517	Tb927.11.10960	hypothetical protein, conserved										
518	Tb927.11.8800	hypothetical protein, conserved										
519	Tb927.5.2530	hypothetical protein, conserved			2			2				
520	Tb927.5.3390	ADG1, pseudogene			1			1				
521	Tb927.7.5680	deoxyribose-phosphate aldolase, putative										
522	Tb927.10.6610	chaperone protein DNAj, putative										
523	Tb927.11.9560	oxidoreductase, putative										
524	Tb927.10.3640	hypothetical protein, conserved			7			6				
525	Tb927.6.4070	hypothetical protein, conserved			2			7				
526	Tb927.11.7100	hypothetical protein, conserved						1				
527	Tb927.11.1680	vesicular-fusion protein SEC18, putative										
528	Tb927.8.5860	50S ribosomal protein L17, putative										
529	Tb927.10.14770	protein kinase, putative							2			
530	Tb927.7.4460	hypothetical protein, conserved										
531	Tb927.2.2160	paraflagellar rod component, putative										
532	Tb927.10.360	hypothetical protein, conserved										
533	Tb927.7.1090	hypothetical protein, conserved										
534	Tb927.11.8030	hypothetical protein, conserved										
535	Tb927.8.3050	hypothetical protein, conserved			1			1				
536	Tb927.11.10080	hypothetical protein, conserved										
537	Tb927.11.10210	hypothetical protein, conserved			2			2				
538	Tb927.6.5090	hypothetical protein, conserved			9			12				
539	Tb927.11.460	hypothetical protein, conserved, predicted WD40 repeat protein										
540	Tb927.11.11250	cytosolic malate dehydrogenase										
541	Tb927.6.1550	hypothetical protein, conserved, leucine-rich repeat protein (LRRP), putative			1			1				
542	Tb927.7.540	chaperone protein DNAj, putative										
543	Tb927.7.5320	hypothetical protein, conserved										
544	Tb927.10.14720	peroxin 13			2			1				
545	Tb927.4.1600	hypothetical protein, conserved			1			1				
546	Tb927.9.3370	thioredoxin										
547	Tb927.10.10800	hypothetical protein, conserved			4			4				
548	Tb927.7.3810	hypothetical protein, conserved										

Transmembrane and Signal Pept

549	Tb927.10.7700	ABC transporter, putative			6	1	Y		5		7	1		Y		6	1	Y		6	1	Y	
550	Tb927.8.8120	hypothetical protein, conserved																					
551	Tb927.7.2670	hypothetical protein, conserved,zinc finger protein family member, putative																					
552	Tb927.9.10070	hypothetical protein, conserved																					
553	Tb927.10.15950	TFIID-like protein, putative																					
554	Tb927.5.1160	hypothetical protein, conserved																					
555	Tb927.6.2720	calcium-binding protein, putative																					
556	Tb927.11.15240	small GTPase, putative,ras-related protein rab-2a, putative																					
557	Tb927.10.600	hypothetical protein, conserved																					
558	Tb927.9.15060	rRNA processing protein, putative																					
559	Tb927.9.5040	cAMP-specific phosphodiesterase																					
560	Tb927.8.4500	eukaryotic translation initiation factor 4 gamma, putative																					
561	Tb927.9.5280	hypothetical protein, conserved																					
562	Tb927.2.4090	hypothetical protein, conserved																					
563	Tb927.8.4890	endoplasmic reticulum oxidoreductin, putative,pol-associated gene 1																					
564	Tb927.10.790	vesicle-associated membrane protein, putative,synaptobrevin, putative																					
565	Tb927.11.3240	T-complex protein 1, zeta subunit, putative																					
566	Tb927.11.10170	hypothetical protein, conserved																					
567	Tb927.10.10880	ATP-binding cassette protein, putative,ABC transporter, putative,ATPase, putative																					
568	Tb927.11.2570	hypothetical protein, conserved																					
569	Tb927.8.3150	T-complex protein 1, gamma subunit, putative																					
570	Tb927.11.11160	sodium/sulphate symporter, putative																					
571	Tb927.10.14030	hypothetical protein, conserved																					
572	Tb927.11.2120	hypothetical protein, conserved																					
573	Tb927.11.14960	pumilio/PUF RNA binding protein 7, putative																					
574	Tb927.6.4970	serine/arginine-rich protein specific kinase SRPK, putative,protein kinase, putative																					
575	Tb927.10.14390	hypothetical protein, conserved																					
576	Tb927.9.9940	PACRGB,flagellar component																					
577	Tb927.10.4430	pumilio RNA binding protein PUF1																					
578	Tb927.6.5210	variant surface glycoprotein (VSG, pseudogene), putative,variant surface glycoprotein, frameshift																					
579	Tb927.8.1240	electron transfer flavoprotein-ubiquinone oxidoreductase, putative																					
580	Tb927.10.12260	cytosolic nonspecific dipeptidase, putative,peptidase (M20/M25/M40 family), putative																					