

**Table S1.** Identification of enzymes involved in the synthesis of the sugar nucleotides UDP-NAcGlc and GDP-Man in *P. falciparum* and the piroplasmids *B. bovis*, *T. equi* and *T. parva*. A scheme of these pathways is presented in Fig. S2. In these four parasites, UDP-NAcGlc is used as sugar donor for N-glycosylation. *T. equi*, additionally uses GDP-Man as mannose donor, while all use this sugar nucleotide in the GPI biosynthetic pathway. n.i.: not identified.

Abbreviation	Enzyme activity	<i>P. falciparum</i>	<i>B. bovis</i>	<i>T. equi</i>	<i>T. parva</i>
G6PI	Glucose-6-P isomerase	XP_001348515	XP_001609553	XP_004832357	XP_763365
GFPT	glutamine-fructose-6-phosphate aminotransferase	XP_001347529	XP_001609193	XP_004832549	XP_765503
GNA	Glucosamine-phosphate N-acetyltransferase	n.i.	n.i.	n.i.	n.i.
PAGM	Phosphoacetylglucosamine mutase	XP_001347982	XP_001611201	XP_004832899	XP_765493
UAP	UDP-N-acetylglucosamine pyrophosphorylase	XP_001350157	XP_001609008	XP_004829714	XP_766009
MPI	mannose-6-phosphate isomerase	XP_001349498	XP_001612088	XP_004831384	XP_764516
PMM	phosphomannomutase	XP_001347454	XP_001610907	XP_004833300	XP_766306
MPG	Mannose-1-phosphate guanyltransferase	XP_001348376	XP_001610191	XP_004829279	XP_765205
HK	Hexokinase	XP_966222	XP_001608748	XP_004830220	XP_765570

**Table S2.** Identification of three enzymes participating in the last steps of dolichol synthesis in piroplasmids. CPT: cis-prenyltransferase, homologous to *P. falciparum* PF3D7\_0826400 (XP\_001349261), containing a cis-IPPS Superfamily conserved domain (cd00475). PPRD: polyprenol reductase, homologous to *P. falciparum* PF3D7\_1455900 (XP\_002585471), containing a enoyl-CoA reductase conserved domain (cl28409). DK: dolichol kinase, homologous to *S. cerevisiae* SEC59 (NP\_013726.1), containing a cytidylyltransferase domain (cl21502). CPT, PPRD and DK were annotated as hypothetical proteins or undecaprenyl diphosphate synthase, 3-oxo-5-alpha-steroid 4-dehydrogenase and dolichol kinase, respectively. (\*) SEC59 homologues with no predicted conserved domain, likely due to sequencing mistakes.

Piroplasmid species	CPT	PPRD	DK
<i>B. bovis</i>	XP_001609396	XP_001611318	XP_001610276 (*)
<i>B. bigemina</i>	XP_012770149	XP_012768612	XP_012766127 (*)
<i>B. ovata</i>	XP_028868789	XP_028867790	XP_028867120
<i>B. divergens</i>	Bdiv_019050	Bdiv_019050	Bdiv_014590c
<i>Babesia sp. Xinjang</i>	XP_028871757	XP_028871510	XP_028871448
<i>B. microti</i>	XP_021337407	XP_012649685	XP_021338572
<i>Theileria equi</i>	XP_004832262	XP_004832719	XP_004829343
<i>C. felis</i>	CF004034	CF002172	CF000174
<i>T. parva</i>	XP_763441	XP_762780	XP_765268
<i>T. annulata</i>	XP_955106	XP_955357	XP_952012.1
<i>T. orientalis</i>	XP_009691469	XP_009692625	XP_009690727

**Table S3.** Predicted N-glycosylated proteins of *B. bovis*, T2Bo strain. Proteins predicted to be soluble, extracellular (A) or membrane-bound (B) and to bear one or more Asn-Xaa-Ser/Thr sequons, without Pro at Xaa, with high score ( $\geq 0.75$  probability) are listed.

A					
#Accession	#GI	<i>Babesia bovis</i> T2Bo	Positions of predicted N-glycosylation sites		
XP_001608682.1	156082395	hypothetical protein	80		
XP_001608758.1	156082547	DnaJ domain containing protein	46		
XP_001609070.1	156083172	spherical body protein 3	727	382	303
XP_001609122.1	156083276	ribosomal protein L3 domain containing protein	81		
XP_001609127.1	156083286	hypothetical protein	130		
XP_001609392.1	156083817	hypothetical protein	608	551	374 328
XP_001609486.1	156084005	HAD superfamily hydrolase	236	82	
XP_001609600.1	156084234	hypothetical protein	391	62	
XP_001609647.1	156084328	hypothetical protein	790	259	14
XP_001609701.1	156084436	hypothetical protein	57		
XP_001609719.1	156084472	hypothetical protein	129		
XP_001609721.1	156084476	mac/perforin domain containing membrane protein	28	24	
XP_001609725.1	156084484	hypothetical protein	345	195	
XP_001609800.1	156084634	membrane protein, putative	40		
XP_001610126.1	156085298	subtilisin-like protein	601	289	
XP_001610179.1	156085539	hypothetical protein	362		
XP_001610192.1	156085565	ubiquitin-conjugating enzyme E2	63		
XP_001610234.1	156085649	hypothetical protein	183		
XP_001610252.1	156085685	erythrocyte membrane-associated antigen	154		
XP_001610300.1	156085781	SmORF	47		
XP_001610337.1	156085856	hypothetical protein	692	431	389 223
XP_001610410.1	156086002	hypothetical protein	452		
XP_001610418.1	156086018	hypothetical protein	276		
XP_001610429.1	156086040	cyclophilin	161		

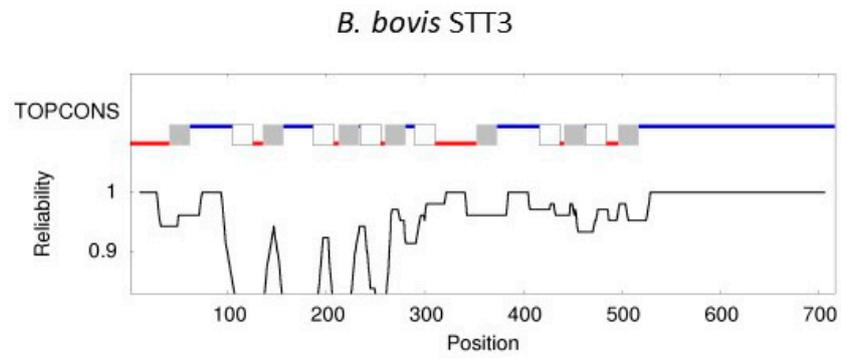
XP_001610441.1	156086064	hypothetical protein	35	19				
XP_001610577.1	156086336	hypothetical protein	30					
XP_001610672.1	156086526	hypothetical protein	564					
XP_001610684.1	156086550	hypothetical protein	674	418	368	305	232	154
XP_001610715.1	156086612	SmORF	108					
XP_001610737.1	156086656	12D3 antigen	229					
XP_001610783.1	156086752	hypothetical protein	39					
XP_001610828.1	156086842	hypothetical protein	90	68				
XP_001610832.1	156086850	protein disulfide isomerase related protein	345					
XP_001610846.1	156086878	hypothetical protein	288	172	52			
XP_001610959.1	156087104	hypothetical protein	374	194	146	72	64	
XP_001611015.1	156087216	hypothetical protein	105					
XP_001611063.1	156087312	hypothetical protein	943	751	580	402		
XP_001611104.1	156087394	hypothetical protein	118					
XP_001611109.1	156087404	WD repeat domain containing protein	588	251				
XP_001611301.1	156087789	LytB protein	46					
XP_001611368.1	156087923	SmORF	59					
XP_001611369.1	156087925	SmORF	74					
XP_001611539.1	156088265	hypothetical protein	195					
XP_001611693.1	156088573	hypothetical protein	410	160	70			
XP_001611710.1	156088607	hypothetical protein	410	160	70			
XP_001611725.1	156088637	tRNA methyl transferase family protein	277	68				
XP_001611782.1	156088751	hypothetical protein	410	240	70			
XP_001612129.1	156089445	ubiquitin family protein	30					
XP_001612214.1	156089615	peptidyl-prolyl cis-trans isomerase, cyclophilin-type f domain containing protein	137	100				
XP_001612255.1	156089697	hypothetical protein	334	309				
<b>B</b>								
<b>#Accession</b>	<b>#GI</b>	<i>Babesia bovis</i> T2Bo	<b>Positions of predicted N-glycosylation sites</b>					
XP_001608872.1	156082776	hypothetical protein	537					

XP_001608971.1	156082974	hypothetical protein	102							
XP_001608980.1	156082992	hypothetical protein	284							
XP_001609412.1	156083857	hypothetical protein	589							
XP_001609788.1	156084610	thrombospondin-related anonymous protein	273							
XP_001609812.1	156084658	p18 protein	785							
XP_001610169.1	156085519	hypothetical protein	930	327	252					
XP_001610553.1	156086288	GCC2 and GCC3 domain containing protein	853	816	497	473	234	229	142	
XP_001610606.1	156086394	hypothetical protein	79							
XP_001610777.1	156086738	p-type ATPase	520							
XP_001610968.1	156087122	hypothetical protein	464	277						
XP_001611503.1	156088193	hypothetical protein	433	27						
XP_001611871.1	156088929	membrane protein	314	169						

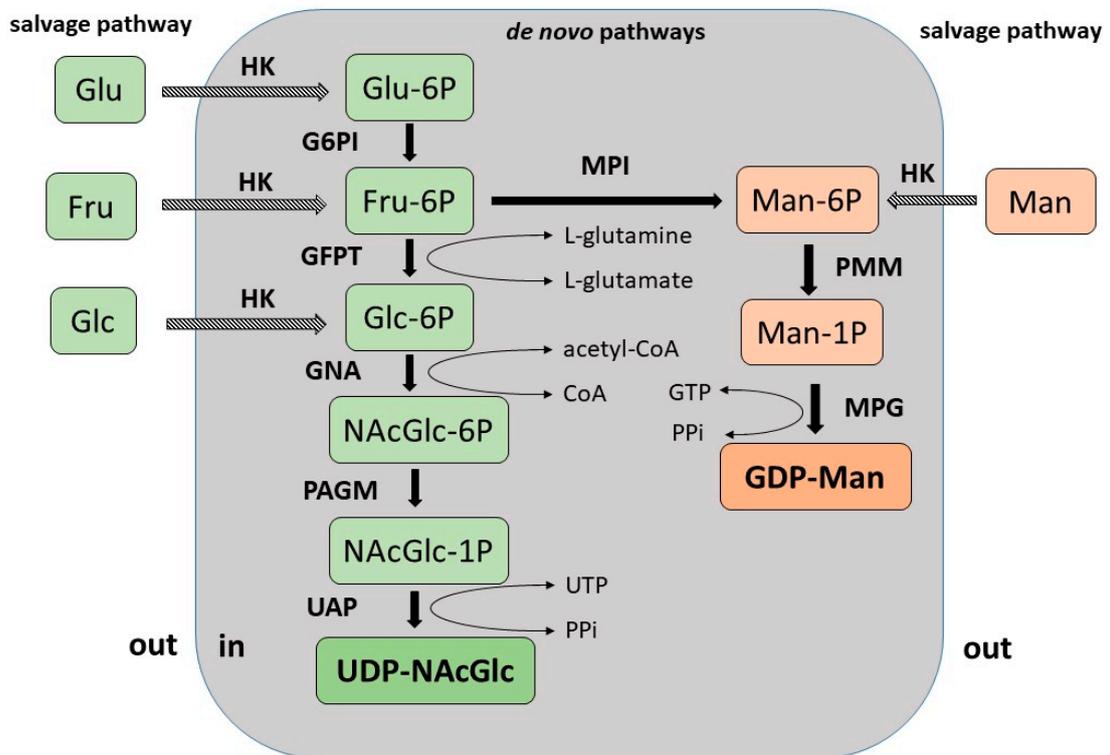
**Table S4.** Transcription levels of N-glycosylation-related genes in *B. bovis* blood and tick stages. Data are expressed as log<sub>10</sub> CPM. As reference, the CPM values for highly transcribed genes in merozoites (MSA-1) and kinetes (Kinete-specific, KS) are shown under “Reference genes”

Process	Gene id#	Genbank annotation of encoded protein	Common name	Log <sub>10</sub> CPM	
				Blood	Tick
N-glycosylation	BBOV_IV000950	N-acetylglucosamine-1-phosphate transferase	Alg7	20.63	14.59
	BBOV_II005640	glycosyl transferase	Alg13	187.84	0.72
	BBOV_IV004090	conserved membrane protein	Alg14	3.38	4.02
	BBOV_II000220	oligosaccharyl transferase STT3 subunit	STT3	41.96	65.29
	BBOV_III003500	hypothetical protein	OST1	44.02	39.59
sugar nucleotide synthesis	BBOV_II000240	glucose-6-phosphate isomerase protein	G6PI	648.05	80.12
	BBOV_IV000250	glucosamine--fructose-6-phosphate aminotransferase	GFPT	493.68	112.73
	BBOV_III000660	phosphoglucomutase	PAGM	12.07	179.66
	BBOV_I003580	UDP-N-acetylglucosamine pyrophosphorylase	UAP	17.29	20.42
	BBOV_III009640	phosphomannose isomerase type I family protein	MPI	45.06	369.44
	BBOV_IV009850	phosphomannomutase	PMM	15.47	1086.53
	BBOV_II006730	mannose-1-phosphate guanyltransferase	MPG	23.83	51.13
	BBOV_I000860	hexokinase	HK	522.38	1166.98
dolichol synthesis	BBOV_IV002310	undecaprenyl pyrophosphate synthetase	CPT	17.97	22.34
	BBOV_III001830	3-oxo-5-alpha-steroid 4-dehydrogenase family protein	PPRD	44.02	39,.89
reference genes	BBOV_I003060	Merozoite surface antigen-1	MSA-1	5162.24	31.97
	BBOV_I002220	hypothetical protein	KS	12.66	243725.20

**Figure S1.** Predicted topology of *B. bovis* STT3. Red: inside; blue: outside; gray boxes; TM helix, IN-OUT; white boxes: TM helix, OUT-IN. “Inside” and “outside” predictions correspond to the cytoplasmic and luminal sides of the ER, respectively.



**Figure S2.** Predicted pathways for the biosynthesis of UDP-NAcGlc and GDP-Man in piroplasmids. *Babesia* s.s. use UDP-NAcGlc as sugar donor for N-glycosylation and GPI biosynthesis, and GDP-Man only for the latter process, while *T. equi* and *C. felis* use both sugar nucleotides in both processes. De novo pathways take place in the cytoplasm (in) starting from glucose-6-phosphate (Glu-6-P) and mannose-6-phosphate (Man-6-P), but salvage pathways for the incorporation of monosaccharides from the extracellular medium (out) are also predicted to take place. Precursors for the synthesis of UDP-NAcGlc and GDP-Man are shown in green and pink boxes, respectively. HK: hexokinase; G6PI: glucose-6-P isomerase; GFPT: glutamine-fructose-6-phosphate aminotransferase; GNA: glucosamine-phosphate N-acetyltransferase; PAGM: phosphoacetylglucosamine mutase; UAP: UDP-N-acetylglucosamine pyrophosphorylase; MPI: mannose-6-phosphate isomerase; PMM: phosphomannomutase; PMG: mannose-1-phosphate guanylyltransferase (adapted from ref. 23). According to the presence of homologous genes in their genomes, piroplasmids use the same sugar nucleotide biosynthesis pathway.



**Figure S3.** Number of predicted N-glycosylation sites in *B. bovis* secreted and membrane proteins. *B. bovis* proteins predicted to be exported or membrane-bound were analyzed for the presence of N-glycosylation sites (Asn-Xaa-Ser/Thr, without Pro at Xaa) with high score (potential >0.75). Only surface exposed sites were counted in the case of membrane proteins.

