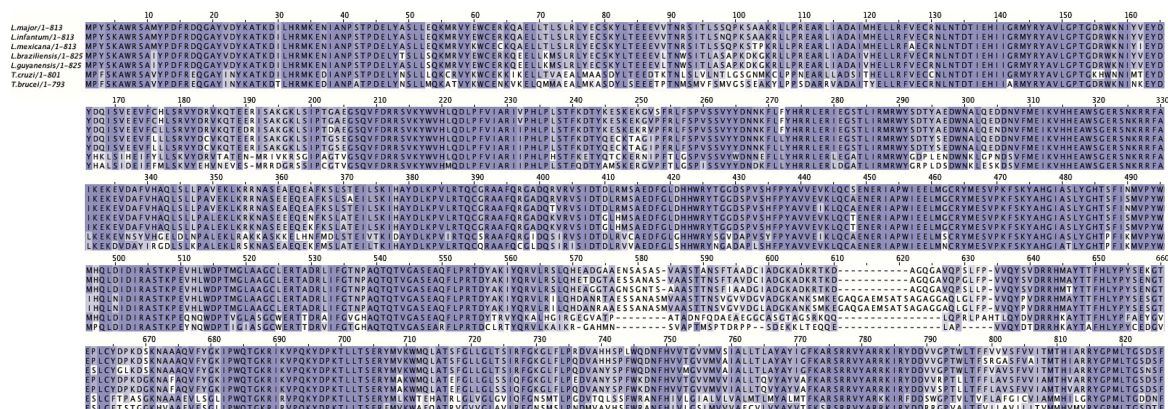


1 Supplementary Information



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3 **Fig. S1. Amino acid sequence comparison of *Leishmania* and *Trypanosoma* VTC4.**

4 A multiple alignment between the different VTC4 amino acid sequences was performed, using
5 ClustalW alignment, within Jalview. The degree of sequence similarity is represented in a dark
6 blue to white color code, indicating high to low sequence conservation of amino acid residues,
7 respectively. *Lmj*: *L. major*; *Lin*: *L. infantum*; *Lmex*: *L. mexicana*; *Lbr*: *L. braziliensis*; *Lgy*: *L.*
8 *guyanensis*; *Tc*: *T. cruzi*; *Tb*: *T. brucei*. The accession numbers of the various sequences used
9 in this study are the following: *L. major*: Q4Q104, GN=LmjF09.0220; *L. infantum*: A4HTX5,
10 GN=LinJ09.0590; *L. mex*: E9AMM1, GN=LmxM09.0220; *L. braziliensis*: A4H5J7,
11 GN=LbrM09_V2.0220; *L. guyanensis*: MF572933; *T. cruzi*: Q4DRN6,
12 GN=Tc00.1047053509023.100; *T. brucei*: Q382V9, GN=Tb11.01.4040.

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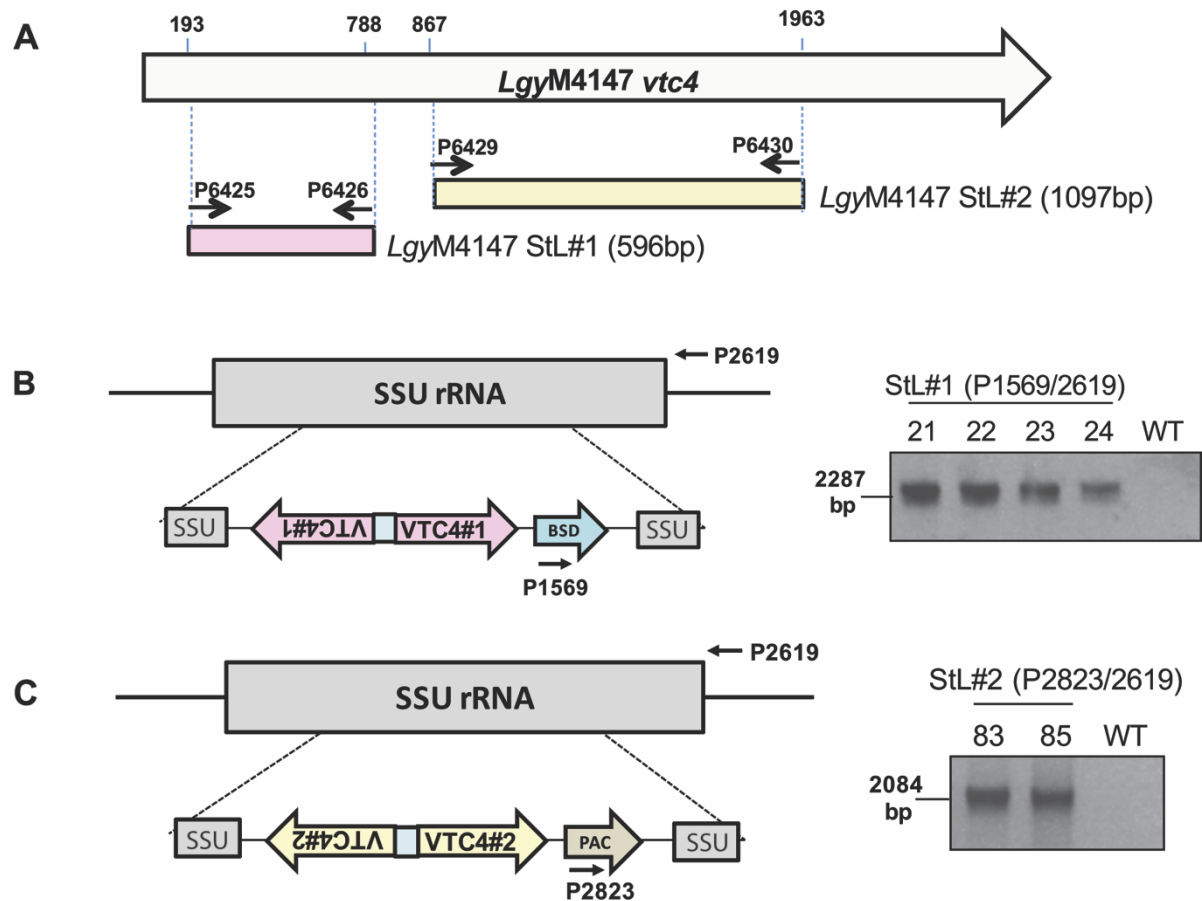


Fig. S2. RNAi interference on *VTC4* in *L. guyanensis* parasites.

(A) Schematic map of StL#1 targeting the 5' end of the gene (193-788 bp) and StL#2 targeting the center (867- 1963 bp) of *L. guyanensis VTC4*. (B, C) Genomic integration of constructs for stem loop formation was verified by PCR on genomic DNA. Amplification at the expected size was detected for primers P1569/P2619 in (B) StL#1 and for primers P2823/P2619 in (C) StL#2 transfectants. Primer sequences are presented in Table S2.

23 **Table S1. *Leishmania* species and clones used.**

Species	Strain
<i>L. major</i>	MRHO/IR/75/ER (IR75) and MRHO/SU/59/P (LV39 clone 5)
<i>L. guyanensis</i>	MHOM/BR/75/M4147 and WHI/BR/78/M5313
<i>L. mexicana mexicana</i>	MYNC/BZ/62/M379
<i>L. donovani donovani</i>	MOHOM/IN/1983/AG83
<i>L. aethiopica</i>	MHM/ET/2008/LDS372
<i>L. braziliensis</i>	MHOM/BR/00/LTB325
<i>L. panamensis</i>	MHOM/ CO/86/1166

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Table S2. Primers used for *L. guyanensis* *VTC4* knockdown generation and for qRT-PCR.

Primer	Sequence (5'→3')	Note
P6425	CGCTAGTCTAGAAagcaggaggagttgctgaaga	under line: XbaI site; bold: <i>VTC4</i> sequences
P6426	CGCTAGTCTAGAAacggagctgacgggcgagaaga	under line: XbaI site; bold: <i>VTC4</i> sequences
P6429	CGCTAGTCTAGAgtggtactcggacacgtactcc	under line: XbaI site; bold: <i>VTC4</i> sequences
P6430	CGCTAGTCTAGAAgtaggggtagaggtggaaggt	under line: XbaI site; bold: <i>VTC4</i> sequences
P1569	TATGATACTAGTATGACCGAGTACAAGCCCAC	forward BSD
P2619	CGACTTTTGCTTCCTCTATTG	Rev SSU
P2823	GGAGGAAGATCTCCACCATGGCCAAGCCTTTGTCTCAAG	forward PAC
fw qRT-KMP11	GCCTGGATGAGGAGTTCAACA	qRT-PCR forward primer for <i>KMP11</i> gene
rv qRT-KMP11	GTGCTCCTTCATCTCGGG	qRT-PCR reverse primer for <i>KMP11</i> gene
fw qRT-Lg-VTC4	TGCGAGAGAAATACGACCCC	qRT-PCR forward primer for <i>VTC4</i> gene
fw qRT-Lg-VTC4	ACGTCCTGTGGCAGAAAGAG	qRT-PCR reverse primer for <i>VTC4</i> gene