MATERIALS AND METHODS

The oligonucleotides used in the cloning and sequencing steps were purchased from Midland and the sequences are available on Table. For the vectors modifications steps, the polymerase chain reaction (PCR) reactions were performed using Taq Platinum DNA Polymerase (Invitrogen®) and the amplified fragments were purified using High Pure PCR Product purification Kit (Roche®). Protein C (*P*rotC) DNA was obtained from DNA oligonucleotide hybridisation. The restriction endonucleases *Asc*I, *Age*I, *Sac*I, *Spe*I, *Sph*I, *Xba*I e *Xho*I were purchased from New England Biolabs®. After cloning the modified plasmids were transfected into the *Escherichia coli ccd*B resistant strain by heat shock. Positive clones were confirmed by colony PCR and plasmids were purified using QIAprep Spin Miniprep kit (Qiagen®). Additional cloning confirmation was done through analysis of the size of the fragments after digestion with specific restriction enzymes. The sequences of the plasmids were confirmed by sequencing performed by the Macrogen facility (Macrogen, Korea).

The oligonucleotides used to clone Mex67 (TcCLB.506127.20), Nup95 (TcCLB.510181.50), Crm1 (TcCLB.511725.150) and TcCLB.506825.40 are shown in Table. After PCR, the DNA fragments were sequenced and cloned into the p*Tc*PTPN-NH 1.1 for Crm1, p*Tc*GFPN-CO 1.1 for TcCLB.506825.40, p*Tc*PTPN-CO 1.1 for Mex67 and Nup95. *Trypanosoma cruzi* were transfected with these plasmids as described in the Supplementary data 3.

The *P*rotC-TEV-*P*rotA-tagged proteins Crm1, Nup95 and Mex67 were analysed by western blot (WB) using the anti-protein A (*P*rotA) antibody (Sigma-Aldrich P3775, 1:40,000 dilution). The localisation of the tagged proteins Crm1, Nup95 and Mex67 was performed by indirect immunofluorescence assays with a rabbit anti-*P*rotA antibody (Sigma-Aldrich P3775, 1:40,000 dilution), 1 h incubation at 37°C. The parasites were then washed with phosphate-buffered saline (PBS) and incubated with Alexa Fluor 488-conjugated goat anti-rabbit IgG or Alexa Fluor 594-conjugated goat anti-rabbit IgG antibodies (Invitrogen, 1:600 dilution) for 1 h. For DNA staining we used 5 µg/mL of 4'-6-diamidino-2-phenylindole for 15 min. Slides were analysed in fluorescence microscope (Nikon E600) and images were captured with a CoolSnap PROcf (Media Cybernetics) camera and analysed with Image Pro-Plus v.4.5.1.22 (Media Cybernetics).

The polyclonal serum raised against the hypothetical conserved protein TcCLB.506825.40 was obtained by four inoculations of 10 µg of the purified 6His-tagged TcCLB.506825.40 in Swiss mice. Each inoculation was performed every 15 days. Ten days after the last inoculation blood was isolated from the mice, the serum obtained by two sequential centrifugations of 10,000 *g* for 10 min and then stored at -20°C. The serum was used at 1:200 dilution to perform WB analysis of *T. cruzi* protein extracts.

To infer the green fluorescent protein (GFP)-tagged TcCLB.506825.40 cellular localisation, transfected epimastigotes were washed in PBS and 1 x 10^6 parasites were added in eight wells teflon microscope slides previously treated with poly-L-lysine for cell attachment, then fixed by incubation for 10 min with 4% paraformaldehyde. The samples were washed with PBS and incubated for 5 min with blue fluorescent dye Hoechst 33342 to stain nucleus and kinetoplast and then washed with PBS. The microscope slides were sealed and images acquired in fluorescence microscope (Nikon E600).

TABLE

Oligonucleotides used for polymerase chain reaction

Primer name	Sequence (5'-3')
Phleo F	CCGCTCGAGACCGGTATGGCCAAGTTGACCAGTGCC
Phleo *Xho*I R	CCGCTCGAGTCAGTCCTGCTCCTCGGC
*Tc*UIR-B F	GGACTAGTCCGGCGCGCCATTGATCTGCGATGCTGTGGACC
*Tc*UIR-B R	GGACCGGTGGTTTCACGTTCCTAACAGTGTG
RP F	GCGAGCTCCACCAGTTTCCTTGCGGG
*Tc*UIR-A R	GCTCTAGAGGTTTCACGTTCCTAACAGTGTGT
Neo *Age*I F	GGACCGGTATGATTGAACAAGATGGATTGC
Neo *Xho*I R	CCGCTCGAGTCAGAAGAACTCGTCAAGAA
Higro F	GGACCGGTATGAAAAAGCCTGAACTCAC
Higro R	GGCTCGAGCTATTCCTTTGCCCTCGGAC
GFP F	GGACTAGTAATGAGCAAGGGCGAGGAGC
GFP R	GGGGCGCGCCTCACTTGTACAGCTCGTCCATGCCG
PTP-CO F	GGACTAGTAGAAGATCAGGTGGATCCTCGTCTTATTG
PTP-CO R	TTGGCGCGCCTCAGGTTGACTTCCCCGCG
*P*rotC F	CTAGTAGAAGATCAGGTGGATCCTCGTCTTATTGATGGGAAAGG
*P*rotC R	CGCGCCTTTCCCATCAATAAGACGAGGATCCACCTGATCTTCTA
Mex67F	**GGGGACAAGTTTGTACAAAAAAGCAGGCTTC**ATGTCAGCTCCATACAAGAAGAAG
Mex67R	**GGGGACCACTTTGTACAAGAAAGCTGGGTC**TGATGCCTGTGGTGTTACCCCT
Nup95F	**GGGGACAAGTTTGTACAAAAAAGCAGGCTTC**ATGTCTATGCTTCACGATGTGTCAG
Nup95R	**GGGGACCACTTTGTACAAGAAAGCTGGGTC**GAGCGCAAACAGCTCTTCTA
Crm1F	**GGGGACAAGTTTGTACAAAAAAGCAGGCTTC**ATGGAGTCCATTTTGGACACAAGT
Crm1R	**GGGGACCACTTTGTACAAGAAAGCTGGGTC**CTATTAAAAAGAGGACGGACGGAAC
TcCLB.506825.40 F	GGCTCCACCATGGGAAACGGCGCCT
TcCLB.506825.40 R	TGGGTGGATYCAATCCTCAAACATCATAAAGAGG

the *att*B recombination sites are shown in bold. The base Y represents a pyrimidine. *att*: attachment; GFP: green fluorescent protein; PTP: *P*rotC-TEV-*P*rotA; RP: ribosomal promoter; *Tc*UIR-A: *Trypanosoma cruzi* ubiquitin intergenic region upstream of cassette Gateway; *Tc*UIR-B: *T. cruzi* ubiquitin intergenic region upstream of resistance marker.